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EFFECT OF HYDROCYANIC-ACID GAS UNDER VACUUM CONDITIONS ON SUBTERRANEAN LARVÆ

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INTRODUCTION

The advisability of fumigating all imported nursery stock at the port of arrival as a requirement for entry has been under consideration for the past five or six years. It is generally recognized that hydrocyanic-acid gas under proper temperature conditions is effective against practically all insects likely to be introduced, provided these pests are neither hermetically sealed in the tissues of the plants nor secreted in the soil around the roots.

Under normal conditions from five to seven million ornamental plants, such as azaleas, rhododendrons, aucubas, conifers, box bushes, bays, etc., are introduced annually with balls of earth around their roots. Needless to say, it is impossible satisfactorily to inspect and safeguard plants of this type without removing the soil from the roots. Inasmuch as practical horticulturists have strongly advised against this procedure on account of its effect on the plants, the only safe course appeared to be fumigation with hydrocyanic-acid gas under vacuum conditions. With this end in view, experiments, listed in Table I, were conducted, in which various dosages and exposures were used, in order that the effectiveness and the degree of safety which might be obtained by fumigating all introduced plants having balls of earth around their roots could be determined.

EXPERIMENTAL PROCEDURE

Owing to the variable amounts of water in the soil surrounding the roots of balled plants at the time of arrival, it was considered advisable to fumigate subterranean larvæ buried at various depths in dry, moist, and soaked soil.[1]

The water content of the moist and of the soaked soil compared favorably with the condition of the soil around the bulk of plants at the time of arrival. Very few plants would survive shipment in soil as free from moisture as that used in the dry experiment. Furthermore, the soil

[1] The soil used consisted of 1 part of manure, 1 part of peat, and 4 parts of loam.

employed was not as compact as is usually the case with the average balled plant.

Through the kindness of Messrs. J. A. Hyslop, J. J. Davis, and W. O. Ellis, of the Bureau of Entomology, United States Department of Agriculture, larvæ of the wheat wireworm (*Agriotes mancus* Say), green June beetle (*Cotinus nitida* Linnaeus), white grub (*Lachnosterna* sp.), and *Popillia japonica* Newman were obtained and fumigated under the following conditions:

All larvæ were exposed to the gas in potting soil, previously described, contained in 3-inch flowerpots, with the exception of those used in experiments 8, 9, and 10 (Table I), in which case 5-inch pots were employed. The position of the larvæ varied, being from 1 to 3 inches from the surface of the soil.

After the larvæ had been placed in the soil, the pots were transferred to the fumigation chamber, the door closed, and the required vacuum produced. At this point the gas was generated and allowed to pass immediately to the fumigation chamber. At the completion of the required exposure the gas was removed from the fumigation chamber by drawing a 25-inch vacuum.

In experiments 2 and 3 the larvæ were exposed to the gas for the entire period in a vacuum; in experiment 4 they were exposed to the gas for 30 minutes in a vacuum, and 30 minutes under normal atmospheric pressure; and in experiment 5 they were exposed to the gas in a vacuum for 30 minutes, followed by a pressure of 10 pounds for 30 minutes. With the exceptions noted, all experiments were conducted with a preliminary vacuum of 26 inches for 15 minutes, with exposures under normal atmospheric conditions varying from 1½ to 2 hours.

CHEMICALS EMPLOYED IN THE GENERATION OF HYDROCYANIC-ACID GAS

Sodium cyanid guaranteed to contain not less than 51 per cent of cyanogen and commercial sulphuric acid (about 1.84 sp. gr., or 66° Baumé) were used in all experiments. The cyanid was used in solution prepared by dissolving 4 pounds of sodium cyanid in 1 gallon of water. The formula employed was as follows:

Sodium cyanid in solution	ounces	2½
Sulphuric acid	ounce	1
Water	ounce	1

RESULTS OF THE EXPERIMENTS

Taken collectively the results given in Table I show that the killing of 100 per cent can not be depended on where the larvæ are in balls of earth around the roots of plants, providing a dosage is used which will not injure the stock. Particularly is this true in the case of moist and soaked soil.

TABLE I.—*Results of experiments in the fumigation of subterranean insects with hydrocyanic-acid gas under vacuum conditions*

Experiment No.	Insects fumigated.	Number of larvæ in soil.			Rate per 100 cubic feet.	Exposure.	Temperature.	Results in—		
		Dry.	Moist.	Soaked.				Dry soil.	Moist soil.	Soaked soil.
					Ounces.		°F.			
1	*Lachnosterna* sp.	3	3	3	½	¼ hour, vacuum 26 inches; 1½ hours, normal atmospheric pressure.	78	3 dead	3 dead	1 dead, 2 alive.
1	*Agriotes mancus*	3	3	3	½	do.		do.	do.	3 alive.
2	do.	4	4	4	1	1 hour, vacuum 26 inches	84	4 alive	4 alive	4 alive.
3	do.	5	5	4	1	1 hour, vacuum at start 27 inches; vacuum at completion 23 inches.	84	5 alive	5 alive	Do.
4	*Lachnosterna* sp.	5	5	5	1	½ hour, vacuum 27 inches; ½ hour, normal atmospheric pressure.	75	5 dead	do.	1 dead, 4 alive.
4	*Agriotes mancus*	5	5	5	1	do.		1 dead, 4 alive.	do.	5 alive.
5	*Lachnosterna* sp.	3	3	3	1	½ hour, vacuum 27 inches; ½ hour, pressure 10 pounds.	69	1 dead, 2 alive.	2 dead, 1 alive.	3 alive.
5	*Agriotes mancus*	3	3	3	1	do.		3 alive	3 alive	Do.
6	*Lachnosterna* sp.	3	3	3	1	¼ hour, vacuum 26 inches; 1½ hours, normal atmospheric pressure.	78	3 dead	3 dead	[a]3 dead.
6	*Agriotes mancus*	3	3	3	1	do.		do.	do.	3 dead.
7	*Lachnosterna* sp.	3	3	3	1	do.	74	do.	do.	1 dead, 2 alive.
7	*Agriotes mancus*	3	3	3	1	do.		do.	do.	Do.
7	*Cotinus nitida*	3	3	3	1	do.		do.	do.	2 dead, 1 alive.
8	*Popillia japonica*	5	5	5	1	do.	80	5 dead	5 dead	5 alive.
9	do.			4	1	do.	73			2 dead, 2 alive.
10	*Lachnosterna* sp.			3	1	¼ hour, vacuum 26 inches; 2 hours, normal atmospheric pressure.				3 alive.
10	*Agriotes mancus*			3	1	do.	80			Do.
10	*Cotinus nitida*			3	1	do.				2 dead, 1 alive.
11	*Lachnosterna* sp.			3	1½	¼ hour, vacuum 26 inches; 1½ hours, normal atmospheric pressure.	85			Do.
11	*Agriotes mancus*			3	1½	do.				Do.
11	*Cotinus nitida*			3	1½	do.				3 dead.
12	*Popillia japonica*			4	3	do.	72			4 alive.

[a] Although all the larvæ were dead, the result is not conclusive, since the larvæ when examined were found to be near the top and side of the pot.

As shown in experiments 2 and 3, satisfactory results are not obtained where the gas is held in the presence of a partial vacuum throughout the entire exposure. It has also been proved that a 30-minute exposure of gas in the presence of a partial vacuum and a 30-minute exposure under normal atmospheric conditions are not effective. The addition of 10 pounds' pressure for 30 minutes in lieu of normal atmospheric pressure yielded practically the same results. It is obvious from Table I that the most satisfactory results were invariably secured where a 15-minute preliminary vacuum was followed by an exposure of one or more hours under normal atmospheric conditions. Especially was this true in the case of dry and moist soil used in experiments 1, 6, 7, and 8, where 100 per cent of the larvæ of *Cotinus nitida*, *Agriotes mancus*, *Popillia japonica*, and *Lachnosterna* sp. were killed. In the soaked-soil tests, however, the results were unsatisfactory.

While there was no notable difference in the resistance to the gas by the various larvæ used, it was apparent that *Popillia japonica* was the most difficult to kill, whereas *Cotinus nitida* was the most susceptible to fumigation.

SUMMARY

(1) The effectiveness of the hydrocyanic-acid gas under the vacuum process is influenced by the water content of the soil.

(2) The death of 100 per cent was not obtained with larvæ in soaked soil at dosages ranging from ½ ounce to 3 ounces per 100 cubic feet of space.

(3) Eliminating the soaked-soil tests, by far the best results were secured where a preliminary 15-inch vacuum preceded an exposure of one and a half hours under normal atmospheric conditions.

(4) Hydrocyanic-acid gas in the presence of a 26-inch vacuum throughout the entire exposure gave negative results with a dosage of one ounce of sodium cyanid per 100 cubic feet and an exposure of 1 hour. An exposure of the gas for one-half hour under 10 pounds' pressure, following a half-hour exposure to a 27-inch vacuum, yielded very indifferent results.

(5) With our present knowledge of vacuum fumigation with hydrocyanic-acid gas, a dosage exceeding 1 ounce of sodium cyanid per 100 cubic feet of space with an exposure of 1½ hours is not recommended for plants in foliage. Inasmuch as all larvæ in soaked soil were not killed with dosages varying from ½ ounce to 3 ounces per 100 cubic feet of space, fumigation at the port of entry with a dosage which will not injure the plants can not prevent the introduction and establishment of all subterranean pests.

CATALASE AND OXIDASE CONTENT OF SEEDS IN RELATION TO THEIR DORMANCY, AGE, VITALITY, AND RESPIRATION

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INTRODUCTION

For some years the Seed-Testing Laboratories of the Bureau of Plant Industry has been using daily alternating temperatures for the germination of certain seeds. In these alternations the lower temperature is used about 18 hours and the higher temperature about 6 hours of the day. Alternating temperatures are found especially favorable for the germination of the seeds of certain grasses: Johnson grass, *Holcus halepensis* L. (*Sorghum halepensis* Pois.); bluegrass, *Poa pratensis;* and Bermuda grass, *Capriola dactylon* (L.) Kuntze. For Johnson grass 30° C. for 18 hours and 45° C. for 6 hours of the day have been found to be the most favorable alternation. Sudan grass, *Holcus halepensis sudanensis* (Piper) Hitchc. (*Andropogon halepensis sudanensis* Piper), which is closely related to Johnson grass and very similar to it in most respects, germinates readily at constant temperatures.

During the last three years (1916–1918) an extensive physiological study of the behavior of the seeds of Johnson grass and Sudan grass has been made in order to get some light on the reasons for the difference in the requirements of the two regarding alternate temperatures for germination. Incidentally such a study has thrown much light upon delayed germination and the physiology of germination in general. Naturally seeds of several other grasses and of a number of other families have been drawn into the investigation. The present paper reports one phase of this extensive comparative study of the physiology of germination.

MECHANICS OF DORMANCY

A brief statement of the mechanics of dormancy in seeds can profitably be made at this point, since it will throw light on experiments to be reported later. There are two general means by which seeds are rendered dormant even when they have morphologically mature embryos and have all the external conditions necessary for germination.

[1] The writers are under great obligation to the following organizations for supplies of seeds used in this work; To the Office of Forage Crop Investigations for Johnson grass and Sudan grass seeds of various ages, together with data as to time and place of collection; to the Seed-Testing Laboratories for several samples of 23-year-old seeds of *Amaranthus retroflexus;* and to the botanical staff of the State College of Washington for the collections of various species of Amaranthus made at Pullman, Wash.

(1) In one type the embryo is dormant (incapable of growth when it is naked and furnished ordinary germination conditions) and must go through some very fundamental changes preliminary to growth. The changes generally require considerable time—weeks or months. Some experiments already published (*15, 19, 20*)[1] and many unpublished experiments made at the Hull Botanical Laboratory by Mr. W. E. Davis and by Mr. R. C. Rose, together with experiments reported in this paper, show the optimum condition for after-ripening of dormant embryos, as well as some of the changes that occur in the embryo during after-ripening. The seeds of this type that have been studied in some detail are: Various species of Crataegus, peach (*Amygdalus persica*), *Ambrosia trifida*, *A. artemisiaefolia*, and basswood (*Tilia americana*). The optimum conditions for the changes seem to be a temperature of about 5° C., with a good oxygen and water supply. The removal of the carpels or other coat structures hastens the process markedly, and it does so, in part at least, by increasing the water and oxygen supply. The following changes have been noted as after-ripening progresses: Continuous rise in the vigor of the resulting seedling, as manifested by rate of growth and resistance to fungal attack; increase in the amount of water absorbed; increase in total acid; slight (due to buffers) but evident increase of H^+ concentration; increase in catalase activity; and increase in oxidase activity, at least as manifested by action on guaiacum and on chromogens already in the plant.

(2) In the second type of dormancy inclosing structures prevent growth of the embryo by holding out some growth factor or holding in some growth inhibitor. In hard seeds water is entirely excluded. In Amaranthus and Alisma (*15, 16*) an initial rapid water absorption ceases before the imbibitional and osmotic forces of the embryo are satisfied, because the swelling of the seed contents is not sufficient to break the seed coats. In many seeds the coats reduce the oxygen supply below the necessary minimum for germination (*15*).

If freshly harvested seeds of Johnson grass are kept in a germinator at 20° C., they will remain dormant for more than a year and probably for many years. Under this condition they go through a change by which they become less capable of germination at higher constant temperatures (25° or 30° C.) than fresh seeds and very much less so than dry stored seeds of the same collection. The senior writer (*15*) has spoken of this deepened dormancy produced in a germinator furnishing conditions unfavorable for germination as "second dormancy" and has pointed out its rather general occurrence as reported in the literature. Johnson grass seeds in dry storage gradually after-ripen, so that their germination improves both at alternate and constant temperatures.

The dormancy in seeds of Johnson grass is imposed by structures inclosing the embryo (scales, pericarp, and tegmen), for removal of

[1] Reference is made by number (italic) to "Literature cited," p. 172–174.

these structures leads to very prompt and practically complete germination in fresh seeds, after-ripened seeds, and seeds in secondary dormancy.[2] The seedlings resulting in every case are about equally vigorous. This indicates that the embryo itself is not dormant. As yet how the inclosing structures enforce dormancy is not known. It is not by the complete exclusion of water, for the intact seeds absorb water rather readily; nor through reduction of the oxygen supply, for these seeds are indifferent to variations in oxygen pressures varying from one-fifth to five-fold that of the atmosphere; nor to narcotic action of carbon dioxid held in by the coats, as Kidd (*28*) has assumed to be the case for all dormant seeds that absorb water readily, for carbon dioxid in high partial pressures is a good forcing agent for dormant seeds of Johnson grass.

CATALASE IN SEEDS

Catalase is an enzym capable of splitting hydrogen peroxid into water and oxygen. It is universally present in living matter and was supposed to be a property of all enzyms until Loew (*30*) showed it to be a distinct body. There is some question arising as to its real enzymic nature (*1*, *2*).

Its function in the organism is not known. Loew believed that in aerobes it protected the organism against the accumulation of hydrogen peroxid produced in respiration. In anaerobes he assumed that it loosened chemical affinities, aiding splittings, oxidations, and reductions. Others have assigned it protective action against excessive oxidations in the organism by organic peroxids or even an essential part in respiration (*27*, *p. 138-140*). Whatever its function Zieger (*37*) has shown some correlation between catalase content and metabolism in animals, and several workers (*2*, *12*, *13*, *29*, *32*) have shown a rather close correlation between respiratory intensity and catalase activity.

EXPERIMENTAL METHODS

The catalase activity was determined by an apparatus similar to that used by Appleman (*1*). A given weight of seed material, after being ground in a mortar and worked through a piece of bolting cloth of desirable mesh, was shaken up in the bottle of the apparatus with 5 cc. of distilled water. Then 5 cc. of hydrogen peroxid, rendered neutral to phenolphthalein by the addition of *N/10* sodium hydroxid, was placed in the dropping funnel and the bottle and dropping funnel lowered into a water bath at 25° C. After the apparatus with its contents had reached the temperature of the water bath the hydrogen peroxid was dropped into the plant emulsion and the mixture continuously shaken. The

[2] One of the best ways to remove these structures is to pick the caryopses out of the scales and treat them in the air-dry condition for two or three minutes with concentrated sulphuric acid. This treatment is followed by thorough washing, first with a 5 per cent solution of sodium hydrogen carbonate and later with distilled water. In the last washing care should be taken to rub away all the carbonized material.

amount of oxygen delivered in any given time was read in the gas burette. The amount of ground material used for each experiment varied from 0.5 to 0.025 gm., depending upon the catalase activity of the tissue. In general 0.14 gm. gave a delivery rate suitable to the size of the apparatus, and except when otherwise stated that is the amount used for each determination in this paper. Bolting cloth of 70 to 80 mesh to the inch was found most desirable. This degree of pulverizing gave the maximum activity for Johnson grass, although finer grinding (100 mesh to the inch) gave somewhat higher activity for the clover.

Dioxogen (H_2O_2 12 V.), of the Oakland Chemical Co., was used almost exclusively in these experiments, but two other brands of hydrogen peroxid were also tried, peroxid of hydrogen, 3.10 per cent, of the Oakland Chemical Co., and hydrogen peroxid, 2.7 per cent, of the J. T Baker Chemical Co. The Bureau of Chemistry furnished the following pharmacopeial analyses of these peroxids:

Peroxid of hydrogen (Oakland Chemical Co.)

Available H_2O_2	2.77 per cent.
Nonvolatile matter in 20 cc	0.021 gm.
Acidity, 25 cc	1.4 cc. *N/10*.
No preservative detected.	

Hydrogen peroxid, C. P. (J. T. Baker Chemical Co.)

Available H_2O_2	2.92 per cent.
Nonvolatile matter, 20 cc	0.036 gm.
Acidity, 25 cc	1.77 cc. *N/10*
Acetanilid (declared 1 part in 7,000)	1 part in 18,000.

Dioxogen (Oakland Chemical Co.)

Available H_2O_2	3.63 per cent.
Nonvolatile matter, 20 cc	0.011 gm.
Acidity, 25 cc	0.9 cc. *N/10*.
No preservative detected.	

It is interesting to compare the percentage of H_2O_2 found in these peroxids by the pharmacopeial method with the percentages calculated from the amount of gas delivered upon adding an excess of seed catalase. In the determinations with catalase 2 gm. of the unneutralized peroxid was diluted with 3 cc. of distilled water, and to this was added an emulsion of 1.5 gm. of powdered crimson-clover seeds in 5 cc. of water. The gas delivered was reduced to standard pressure and temperature and its weight calculated upon the assumption that it was all oxygen. From this the percentage of H_2O_2 in the peroxids was then calculated. The values given in every case are the average of two or more closely agreeing determinations. These percentages are shown in Table I.

It is seen that the catalase determinations are somewhat larger in every case and that the percentage excess is about constant. This discrepancy is probably due to a consistent error in one or the other of

the series of determinations, but the possibility remains that the catalase may split some of the H_2O_2 into hydrogen and oxygen. On the basis of the first and the more likely assumption it seems probable that catalase gives a complete decomposition of H_2O_2.

The biologist needs to know the concentration of the hydrogen peroxids he uses, whether they are used as sterilizing agents, forcing agents for dormant seeds, or for catalase determinations. He will find the catalase method sufficiently accurate and easily run with materials at hand. The determination can be made without neutralizing the acidity of the peroxids, even when it is rather high. The great excess of the plant powder used (probably acting as a buffer), along with the dilution of the peroxids, sufficiently counteract the inhibiting effects of the acids to give complete decomposition.

We have found dioxogen a desirable brand of hydrogen peroxid for catalase determinations and for use as a forcing agent for dormant seeds. It bears a rather high percentage of H_2O_2. The concentration runs almost constant for different bottles of the same lot (date cut into the label) and for bottles of different lots so far as our examinations have gone. Its acidity is also low and about constant.

TABLE I.—*Percentage of H_2O_2 in hydrogen peroxids*

Source of material.	Method of determination.	
	Excess of catalase.	Pharmacopeial.
Peroxid of hydrogen, Oakland Chemical Co.	2.77	2.68
Hydrogen peroxid, J. T. Baker Chemical Co.	3.02	2.92
Dioxogen, Oakland Chemical Co.	3.75	3.62

Before using a bottle of dioxogen we always determined the percentage of H_2O_2 in it. We also neutralized portions of it to phenolphthalein as they were to be used. The 5 cc. employed in each catalase determination is capable of delivering about 65 cc. of oxygen at the temperature of the experiment. The concentration of catalase and time was so adjusted that in general not more than one-half the oxygen was delivered in an experiment, thus giving a great excess of hydrogen peroxid.

EXPERIMENTAL WORK

EFFECT OF ACIDITY

Various workers (*1*, *30*) have mentioned the sensitiveness of catalase to acids and have pointed out the fact that maximum activity and minimum destruction occur in a neutral or slightly alkaline medium.

Appleman (*1*) seems to consider the plant material the main source of destructive acids. In our work with seeds the hydrogen peroxids are the main source of injurious acids. Without neutralization the two more acid hydrogen peroxids mentioned above give (with Johnson grass) only about one-third as much catalase activity as does the less acid dioxogen. When all these peroxids are neutralized or an excess of calcium carbonate is used, the three give more nearly equal catalase activity. The more acid ones are still somewhat below the dioxogen, due either to lower concentration or other inhibitors. Table II shows the effect of the reaction of the dioxogen upon the catalase activity of Johnson grass.

TABLE II.—*Catalase activity as modified by the reaction of the dioxogen*

[Johnson grass dry-stored 1 year. 70-mesh bolting cloth. 0.1 gm. of meal. 0.9 cc. of *N/10 sodium hydroxid* needed to neutralize 25 cc. of dioxogen to phenolphthalein]

Amount of *N/10* sodium hydroxid added.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
One-half amount needed to neutralize	4. 1	7. 5	9. 9	13. 9
Full amount needed to neutralize	4. 2	7. 5	10. 0	14. 1
Twice amount needed to neutralize	4. 3	7. 8	10. 0	13. 9
Three times amount needed to neutralize	4. 1	7. 6	9. 7	12. 8
None	1. 9	4. 3	5. 6	7. 9
Excess of calcium carbonate	4. 1	7. 8	10. 2	13. 9

Table II shows that the natural acidity of dioxogen reduces greatly the catalase activity of Johnson grass, but that an addition of sodium hydroxid from one-half of the amount needed for neutralization to phenolphthalein to twice the amount needed for such neutralization gives maximum activity. Dioxogen that is half neutralized to phenolphthalein is still acid to neutral red. As is well known, neutral red turns practically at the neutral point ($H^{+} = 10^{-7}$), while phenolphthalein turns at a point that is distinctly basic ($H^{+} = 10^{-9}$). The filtrate from the dioxogen-catalase mixture (the dioxogen being neutral to phenolphthalein) in the above experiments is basic to neutral red, while the filtrate from the emulsion of seed material is acid to neutral red. From all the facts here reported it appears that catalase of Johnson grass is rather sensitive to acids, but that it gives maximum activity in a considerable range of reaction from very slightly acid to rather markedly basic.

Table III shows the effect of the reaction of the dioxogen upon the catalase activity of a number of seeds. An examination of the table will show that unneutralized dioxogen inhibits in all and that the magnitude of the inhibition falls as the amount of powder increases. The plant material apparently acts as a buffer. In the after-ripened peach seed when 0.2 gm. of the seeds is used there is practically no inhibiting, while it is very marked with 0.05 gm. These relations for the peach are well shown by the curves in figure 1.

From these results it is evident that one must look after the reaction of the hydrogen peroxid in measuring the catalase of seeds. In his work with the potato tuber Appleman (*1*) maintained a neutral reaction by adding an excess of calcium carbonate. He showed that the emulsion of ground tuber either contained or developed sufficient acid to injure greatly the catalase after standing for a number of days and that this injury could be avoided by grinding the tuber with an excess of calcium carbonate. It is probable, however, that the low catalase activity of newly-ground tubers not treated with calcium carbonate was partly, if not mainly, due to the acidity of the hydrogen peroxid used.

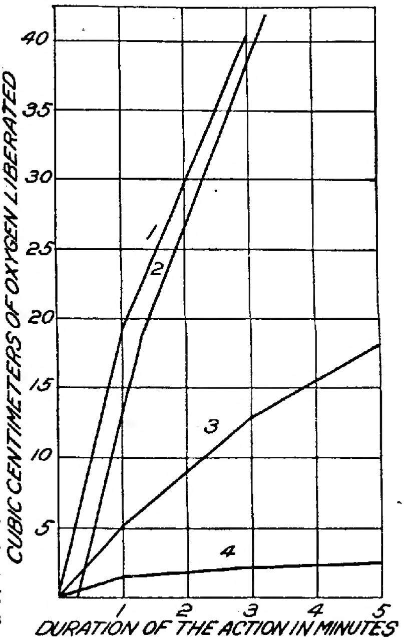

FIG. 1.—Graphs showing the effect of the acidity of dioxogen upon the catalase activity of after-ripened peach seeds; also the buffer effect of the seed material; 1, 0.2 gm. of seed material, dioxogen neutralized; 2, 0.2 gm. of seed material, dioxogen not neutralized; 3, 0.05 gm. of seed material, dioxogen neutralized; 4, 0.05 gm. of seed material, dioxogen not neutralized.

DEGREE OF PULVERIZATION

The degree of pulverization has considerable effect upon the activity of catalase. As Table IV shows, Johnson grass gives maximum activity with medium fineness of the powder (70–80-mesh sieve) and very much less activity with fine grinding (200-mesh sieve), as well as with much coarser meal (40-mesh sieve). Crimson clover gives maximum activity

TABLE III.—*Effect of the reaction of the dioxogen upon the catalase activity of various seeds; also the buffer effect of the seed material*

[0.9 cc. of *N*/10 sodium hydroxid neutralizes 25 cc. of dioxogen to phenolphthalein. 100-mesh bolting cloth for clover; 70-mesh for other seeds.]

Seed used.	Powder used.	*N*/10 sodium hydroxid added per 25 cc. of dioxogen.	Oxygen liberated after— 1 min.	3 min.	5 min.
	Gm.	*Cc.*	*Cc.*	*Cc.*	*Cc.*
Sudan grass	0.1	0.45	3.4	6.9	9.2
		.9	3.8	9.9	14.0
		.0	1.5	4.7	7.4
Amaranthus blitoides	.2	.9	6.1	11.8	14.7
		.0	4.8	9.0	10.9
Amaranthus retroflexus	.1	.9	4.9	9.0	11.1
		.0	2.4	4.5	5.6
	.2	.45	9.5	17.3	21.3
		.9	9.6	17.7	21.6
		2.7	9.0	16.7	20.2
		.0	6.7	12.5	15.0
Peach, dry-stored	.2	.9	.9	2.6	4.1
		.0	.9	2.5	4.0
Peach, after-ripened	.2	.9	18.7	40.5	....
		.0	18.3	41.8	....
Do	.05	2.7	5.7	15.7	21.6
		.9	5.2	12.8	18.1
		.0	1.5	2.2	2.5
Crimson clover (*Trifolium incarnatum*)	.05	.45	12.0	15.1	15.9
		.9	15.0	22.6	24.3
		2.7	15.8	23.8	25.9
		4.5	15.3	22.7	23.9
		.0	6.1	8.1	8.8

with 100-mesh and very nearly maximum with 200-mesh, but much reduced activity with 40-mesh. Agitation of enzyms generally causes degeneration. The fall in activity with finer pulverization may be due to the denaturing of the catalase by excessive mechanical manipulation in grinding and working the powder through the very fine bolting cloth.

TABLE IV.—*Effect of the degree of pulverizing upon catalase activity*

[Bolting cloth used: 70, 80, 100, and 200 mesh to the inch. The sieve used had circular holes 0.610 mm. in diameter]

Seed used and weight of powder.	Mesh of sieve per inch.	Oxygen liberated after— 1 min.	3 min.	5 min.	10 min.
		Cc.	*Cc.*	*Cc.*	*Cc.*
Johnson grass, 0.14 gm	200	3.1	6.8	9.2	13.9
	100	3.7	9.0	11.9	16.7
	80	4.9	10.5	14.4	20.3
	70	4.9	10.8	14.6	20.8
	(a)	3.1	7.3	10.5	15.2
Crimson clover, 0.07 gm	200	15.9	20.9	21.9	22.3
	100	16.1	22.1	23.6	24.7
	70	15.0	21.0	22.5	24.0
	(a)	7.5	12.1	14.5	17.4

[a] 0.610-mm. holes.

Table V shows the diameter of the cells of various parts of the two seeds mentioned in Table IV; also the pore diameter of some of the sieves used. Maximum activity is obtained when the average diameter of the sieve mesh is several times the diameter of the cells. The greater injury from fine pulverizing in Johnson grass may be due in part to the larger average cell diameter. Johnson grass catalase may also be relatively sensitive to mechanical agitation, as later sections show it to be to heat and aging effects. The endosperm of grasses has extremely low catalase activity, so it is left out of consideration in this connection. It is evident from the facts mentioned in this section that attention must be given to uniformity and fineness of pulverization.

TABLE V.—*Diameter of cells of various parts of seeds of Johnson grass and crimson clover*

Material.	Cell or mesh diameter.
	μ
Crimson clover:	
Cells of cotyledons	16– 40
Hypocotyl, except small cells of plerome	15– 30
Johnson grass:	
Scutellum, except elongated enzym layer	33– 66
Coleoptile and coleorhiza same, except small primordial cells	16– 33
200-mesh bolting cloth	33– 75
100-mesh bolting cloth	140–183
70-mesh bolting cloth	214–250
Sieve with 0.610-mm. holes	610

DEGENERATION OF THE POWDER

The powder ready for catalase determination degenerates rather rapidly when stored in a desiccator over quicklime. Table VI shows typical data for Johnson grass, the only species tested. This degeneration may be due in part to excessive drying, but it is probably in the main an aging change. A later section shows that there is a slow time degeneration in intact seeds. The morphological integrity, however, seems to secure very slow degeneration. From the results reported in this section it is evident that it is best to grind and screen the seed material just previous to making the catalase determinations.

TABLE VI.—*Degeneration of catalase in the powder of Johnson grass seeds*

Period of storage of powder.	Oxygen liberated after— 5 min.	Oxygen liberated after— 10 min.
	Cc.	*Cc.*
1 day	13.1	18.6
54 days	4.0	5.8

In his work on plant oxidases Bunzell (*11*) frequently uses powdered plant material, but he has apparently made no statement as to the degeneration of the oxidase in it with storage.

VARIOUS ORGANS OF THE SEED

In the grains of grasses there is considerable difference in the catalase activity of different regions of the grain. Tables VII, VIII, IX, and X give data on this point. In Stoner wheat (Table VII) the catalase activity of the embryo is 28 to 29 times that of the endosperm. The complete caryopsis shows very low activity as compared with the embryo and less than twice that of the endosperm. The embryo of this wheat constitutes about 3.3 per cent of the caryopsis. The low catalase activity of the endosperm agrees with the claim of Grüss (*24*, p. *20–34*) and others that it is composed in the main of dead cells except for the aleurone layer. Burlakow (*4*) has found that the respiratory intensity of wheat embryos is about 20 times that of the endosperms. It seems then that in these seed organs high catalase activity parallels high respiratory intensity.

TABLE VII.—*Catalase activity of embryo, endosperm, and caryopsis of Stoner wheat; collected in August, 1917; run on October 5, 1917*

Part of seed used	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Caryopsis less embryo	0.8	1.4	1.7	2.0
Caryopsis	1.3	2.2	2.7	3.5
Embryo	23.3	39.3		
Ratio, embryo to endosperm	29:1	28:1		

With the smaller grass seeds it is an almost endless task to separate enough embryos to make catalase determinations, so in such grasses other methods were used for estimating the activity of various regions. In a fixed hybrid of Tunis grass and sorghum the catalase activity was measured for the caryopsis and the endosperm (Table VIII). The activity of the embryo was calculated from these data and from the fact that the embryo constitutes 9.6 per cent of the caryopsis. Such calculations tally closely with the actual measurements in the wheat, and it is probable they would do so here.

TABLE VIII.—*Catalase activity of the caryopsis and endosperm of a fixed hybrid of Tunis grass and sorghum seeds freshly harvested*

Region of seed used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Caryopsis less embryo	4.4	9.8	13.4	19.0
Caryopsis	11.3	22.2	29.2	37.9
Calculated for embryo	65.7	112.5	133.1	170.1
Ratio, embryo to endosperm	16.4:1	11.6:1	10:1	9:1

In Sudan grass the caryopsis was divided into a distal and proximal portion by cutting it crosswise just distal to the embryo. The proximal embryo portion was somewhat larger than the distal endosperm portion. As shown in Table IX, the embryo portion is more than twice as active as the complete caryopsis and several times as active as the endosperm end. Here, again, the embryo has very high catalase activity in comparison with the endosperm.

TABLE IX.—*Catalase activity of the distal and proximal ends of the caryopsis of Sudan grass collected at Khartum, Africa (1911?). Immature and mature grains separated with a vertical air-blast separator*

Sort of grain used.	Portion of caryopsis used.	Oxygen liberated after—			
		1 min.	3 min.	5 min.	10 min.
		Cc.	*Cc.*	*Cc.*	*Cc.*
Immature grains	Endosperm end	1.3	2.9	3.9	5.6
Do	Embryo end	17.5	32.0	38.5	....
Do	Complete	6.4	12.5	16.3	22.2
Mature grains	Endosperm end	.8	1.8	2.4	3.5
Do	Embryo end	7.8	15.9	20.9	28.0
Do	Complete	3.8	7.5	10.1	14.2

Table X shows the relative activity of caryopses, the bracts inclosing them, and the sterile florets of Johnson grass. As one would expect, the nonliving and nonfunctioning parts show low catalase activity. They likely also show very low respiratory activity. The data of this section show that the catalase activity of the various organs of the grains of grasses parallels the physiological activity of these organs. The catalase in the endosperm, caryopsis bracts, and sterile florets may be a residuum of previous physiological activity. This seems to be the case, at least with the last two organs mentioned, for in them catalase activity shows an enormous fall with one year of dry storage. In this time it falls to one-seventh, or even one-tenth, its activity in the fresh but well-ripened seed. A later section shows a time fall in the catalase of the caryopsis of grasses, but the rate of fall in this is much slower.

TABLE X.—*Catalase activity of various organs in a sample of Johnson grass seeds*

Condition of plant.	Part of plant used.	Oxygen liberated after—	
		5 min.	10 min.
		Cc.	*Cc.*
1 year of storage	Caryopses	13.0	18.4
Do	Bracts surrounding caryopses	.5	.7
Do	Sterile florets	.6	....
Freshly harvested but mature Johnson grass.	Caryopses	23.5	32.8
Do	Bracts covering caryopses	3.5	5.9
Do	Sterile florets	6.3	8.6

MATURITY OF THE GRAINS

The catalase activity of the grains of grasses is determined to a large degree by their maturity at the time of harvest. The immature grains have much higher activity than the mature ones. This holds for Sudan grass, as shown in Table VIII. This table also shows that the higher activity of immature grains is not lost with thorough drying, but that it is maintained after years of dry storage.

Since the immature caryopses are much smaller than the mature ones, the question naturally occurs, Is the activity per caryopsis about the same in mature and immature seeds? Table XI gives data that answers this question. In this experiment 10 seeds were used in each determination.

TABLE XI.—*Catalase activity of an equal number of mature and immature seeds of Sudan grass collected at Khartum, Africa (1911?)*

Caryopses used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min
	Cc.	*Cc.*	*Cc.*	*Cc.*
10 immature, 0.05 gm.— of powder	2.1	4.0	5.3	7.2
10 mature, 0.07 gm.+ of powder	2.1	4.1	5.5	7.7

It is evident that the activity per caryopsis, whether mature or immature, is about equal. It is not known whether the embryo (the region of main activity) makes up a greater percentage of the caryopsis in the immature ones or whether there is about constant catalase activity per embryo regardless of size and maturity. The endosperms may also be more active in the immature caryopses.

Table XII shows the catalase activity of immature and mature caryopses of Johnson grass about two weeks after it had been harvested. The caryopses were removed from the bracts in a bunch of grains that ranged from medium to thorough maturity, and divided into two lots: Mature (large, plump, dark-brown caryopses), and immature (small, somewhat wrinkled, pink to light-brown caryopses).

TABLE XII.—*Catalase activity of mature and immature caryopses of Johnson grass; collected on September 14, 1917; run on September 28, 1917*

Caryopses used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Immature	11.5	22.4	29.6	40.6
Mature	8.9	17.5	23.5	32.8

Newly harvested seeds of *Amaranthus retroflexus* show a similar relation between the catalase activity of immature and mature seeds. The data for this seed are given in Table XIII.

TABLE XIII.—*Catalase activity of mature and immature seeds of Amaranthus retroflexus; collected on September 14, 1917; run on September 24, 1917*

Caryopses used.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Mature	7.9	13.9	16.5	19.7
Immature	12.5	25.0	30.6	36.7

From the data of this section it is evident that in comparing the catalase activity of different lots of seeds one must be sure of the equal maturity of the lots compared. The experience of the writers shows that this can be approximated by the careful use of the vertical air-blast separator.

EFFECT OF DRYING ON CATALASE ACTIVITY OF SEEDS

In the case of peach and Johnson grass, drying the seeds after they have been in the germinator reduces the catalase activity very markedly in the first and noticeably in the second. Table XIV shows the reduction when slices of after-ripened peach seeds are dried rapidly before being ground. A considerably larger percentage of fall occurs in seeds that have been in the germinator for the same time at 20° or 25° C., although they have much lower absolute catalase activity, owing to their non-after-ripened condition. Intact seeds that have been dried for several days in the laboratory show a still greater percentage of fall. This also occurs where seeds are taken from the fresh fruit and allowed to dry.

TABLE XIV.—*Effect of drying seeds on catalase activity*

[Peach seeds (carpel removed) after-ripened at 7° C. for 54 days: one lot ground and used without drying, the other cut into thin slices and dried 3 hours before an electric fan previous to grinding. 0.1 gm. of material per run]

Sample used.	Oxygen liberated after—		
	1 min.	3 min.	5 min.
	Cc.	*Cc.*	*Cc.*
Ground wet	10.3	26.4	38.3
Ground after drying	3.0	9.1	14.5

Table XV shows the considerable fall in catalase activity of recently harvested Johnson grass caused by a short sojourn in a germinator, also the less considerable additional fall due to drying. It seems that the

catalase activity of these seeds can be greatly reduced by repeated subjection to germinative conditions followed by drying.

TABLE XV.—*Effect of drying on catalase activity of Johnson grass; collected on September 22, 1917; run on October 22, 1917*

Treatment of seed.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Stored dry	6.3	13.7	19.0	27.4
In germinator 14 days at 20° C	4.9	10.2	14.2	21.2
In germinator 14 days at 20° C., then dried 2 days	4.3	9.1	12.6	18.5

Table XVI shows the fall in catalase produced by drying a sample of Johnson grass that has low activity, due to a year's sojourn in a germinator, at 20° C. The absolute fall is low, but the percentage fall rather large.

TABLE XVI.—*Effect of drying on catalase activity of Johnson grass in germinator for 1 year at 20° C.*

Treatment of seed.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Ground imbibed	1.5	2.3	2.7	3.4
Ground after drying 5 days	.8	1.6	2.2	3.1

In the after-ripened seed of basswood the catalase activity seems to rise with a few days' drying. This rise is only apparent, however, for the pulverized material from imbibed seeds contains more moisture when weighed than does the material from dried seeds, while the same weight was used for the determinations in the two cases. For the same reason the effect of drying, in both peach and Johnson grass, was much greater than the figures above indicate. It was not considered worth while, however, to go to the considerable trouble of correcting for the differences in moisture in the dried and undried material, for it would make a difference only in the magnitude and not in the direction of the results.

RELATION OF AGE AND VITALITY OF SEEDS TO CATALASE ACTIVITY

A number of workers (*5, 6, 7, 8, 35*) have shown that the oxidizing and digestive enzyms of old seed still persist after the seeds have completely lost their vitality, but it seems that these enzyms also gradually degenerate with age, although their complete degeneration follows much later than the complete loss of vitality.

Table XVII shows the relation of catalase activity to age and vitality in the seeds of Johnson grass, Sudan grass, and *Amaranthus retroflexus*.

TABLE XVII.—*Relation of catalase activity and age in dry-stored seeds*

[Only mature seeds used. Catalase determinations made between Sept. 26 and Oct. 5, 1917]

Kind and source of seeds.	Date of collection.	Oxygen liberated after—				Germination temperature.	Percentage of germination in—			
		1 min.	3 min.	5 min.	10 min.		3 da.	5 da.	7 da.	15 da.
		Cc.	*Cc.*	*Cc.*	*Cc.*	°*C.*				
Johnson grass:										
Arlington Farm, Va.	1917	6.3	13.7	19.0	27.4	25-40	......	......	......	54
Do.	1916	4.3	8.6	11.4	16.0	25-40	......	......	......	98
Fort Worth, Tex.	1911	3.2	6.5	8.5	11.6	25-40	......	......	......	79
Sao Paulo, Brazil	1908	1.6	3.1	4.2	6.0	25-40	......	......	......	00
Bushnell Co., St. Louis, Mo.	1905	1.7	3.3	4.4	6.3	25-40	......	......	......	19
Soudan grass:										
Sherman, Tex.	1916	4.2	9.1	12.4	17.3	25	......	99	......	......
Khartum, Africa	1911	3.8	7.5	10.1	14.2	25	......	88	......	......
Khartum, original importation	1908	2.0	4.2	5.8	8.2	25	......	98	......	......
Amaranthus retroflexus:										
Pullman, Wash.	1917	8.7	18.1	22.7	26.8	40	99	100	100	......
Arlington Farm, Va.	1917	8.0	13.9	16.7	19.8	40	30	91	100	......
Chicago, Ill.	1917	8.3	16.8	20.7	24.9	40	37	100	100	......
Do.	1915	8.4	16.5	20.9	24.8	40	94	100	100	......
Do.	1914	7.9	15.7	19.2	22.3	40	100	100	100	......
Allenton, Mo.	1894	8.5	13.8	15.5	18.5	40	00	00	00	......
Fort Collins, Colo.	1894	6.3	12.5	15.2	17.0	40	00	00	00	......
East Lansing, Mich.	1894	8.7	18.1	22.7	26.8	40	00	00	00	......
Amaranthus graecizans:										
Pullman, Wash.	1917	4.4	8.5	10.5	13.0	40	12	85	100	......
Amaranthus blitoides:										
Pullman, Wash.	1917	3.5	7.3	9.1	11.0	40	3	26	58	......

In Johnson grass the fall evidently begins with harvest and continues for an indefinite time. The degeneration of catalase seems most rapid during the first year and slows down considerably during later periods. The same thing apparently happens in Sudan grass, but data are lacking for the 1917 crop. In both a very considerable fall in catalase activity occurs, with little or no fall in vitality. There is also considerable catalase activity when seeds fail to germinate at all. In the 9-year-old Sudan grass the percentage of germination is still very high (98 per cent), although the catalase activity has fallen to less than one-half that of the 1-year-old, and probably to less than one-third that of the freshly harvested. In the 9-year-old seeds there is somewhat lower vigor, as shown by the rate of growth of the seedling and the predisposition of the seed to fungus attack. In Sudan grass the catalase activity seems to be an excellent indicator of age. It is apparently a better indicator of age than it is of vitality. The same relation holds for Johnson grass, in which the catalase activity falls continuously with age except for the poor crop of 1908, which shows a slightly lower activity than the 1905 crop.

Figure 2 shows graphically the change in catalase activity and the percentage of germination with aging in Johnson grass seeds. The rather great irregularity in these curves is probably largely due to the diversity of source and handling of the several crops. In general, however, the catalase curve is concave upward while the vitality curve is convex. The rise in germination during the first year is due to after-ripening and not to increased viability, for treatment of the new seeds raised their germination to practically 100 per cent. It is interesting to note the similarity of these curves to the catalase viability curves in heated seeds given on p. 154. This similarity strengthens the coagulation conception of age degeneration (p. 154).

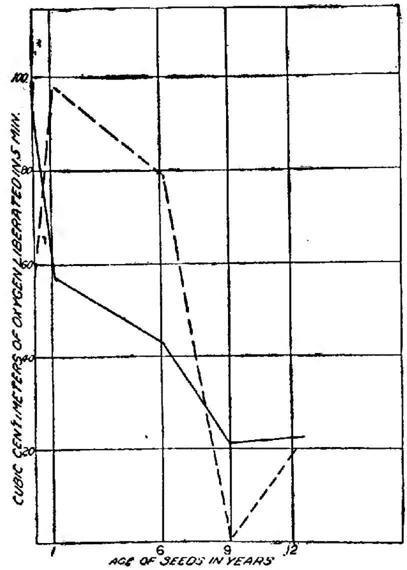

FIG. 2.—Graphs showing changes in viability and catalase activity in Johnson grass, caused by aging: Solid line=catalase; broken line=viability.

Catalase activity might easily be used as a method of estimating the age of seeds. If such were done, the following precaution would be necessary: The test must be compared with a crop of seeds of the same variety and of known age and like maturity, to serve as a standard. It could be applied only to seeds that have markedly time-labile catalase. It must be known that the seeds have gone through no drastic catalase-destroying condition, such as subjection to high temperatures or repeated wetting and drying. Before one could apply this method to any particular kind of seed he would have to know in detail the behavior of its catalase, and the method would show its main value when applied in conjunction with viability tests.

Practically all grass seeds go through a so-called after-ripening period following harvest. Air-dry storage or even storage in a drier condition seems to be a good, if not the best, condition for after-ripening. After-ripening is marked by improvement in rate and percentage of germination. The period of after-ripening is transient and is accompanied by only moderate improvement in germination in most of our readily ger-

minating grain grasses. In other grasses that are markedly resistant to germinating conditions the period is longer and the increase in germinative capacity very marked (*3, 21*). Even in the grasses that ordinarily germinate readily, the dormancy may be deepened and the after-ripening rendered slow and important by certain conditions during ripening. This is true of "rain barley" (*36*) (barley ripening during rainy weather), and to a degree of "frosted grains" (grains frosted during the early stages of ripening) (*34, p. 436*). So far as studied, the dormancy in grass seeds seems to be imposed mainly by coat structures, and there is no evidence of rise in vigor of the embryo with after-ripening, as there is in peach and other seeds in which the embryos have a self-imposed dormancy. It is also interesting to note that in the grasses there is no rise but a very considerable fall in catalase activity with after-ripening, while, as shown in a later section, there is a great rise in catalase activity with after-ripening in seeds in which the embryos themselves determine the dormancy.

There is no evidence that the catalase of Johnson grass differs essentially either in amount or time lability from that of Sudan grass, although the seeds of the former are very refractory to germination conditions, and the latter respond readily.

In the seeds of *Amaranthus retroflexus* there is considerable variation in the catalase activity of the several crops studied, but there is no regular fall with age or even viability of the seeds. The catalase of this seed seems to be far more nearly time stable than is that of the grasses studied.

Crocker and Groves (*17, 23*) have offered considerable evidence for the conception that age degeneration of seeds is due to a time-temperature denaturing of certain colloids (probably proteins) of the embryo. There also seems to be a time denaturing of the catalase of seeds, but it does not parallel the time denaturing of the materials essential to viability. The time lability of substances connected with viability may be compared with the time lability of catalase. The former are relatively time-stable in seeds of Johnson grass and Sudan grass (at least for the early period of storage), while the latter is relatively time-labile. In species of Amaranthus the former are relatively time-labile, while the latter is nearly time-stable.

Evidence given in other parts of the paper indicates that catalase activity is more closely correlated with respiration intensity than it is with viability; but the correlation with respiration is evidently not universal, for one can hardly conceive that seeds of *Amaranthus retroflexus* that have died from age still maintain full respiratory vigor.

The slower or lower percentage of germination shown in the 1917 Johnson grass and two lots of the 1917 *Amaranthus retroflexus* seeds is due to the unafter-ripened condition and not to low vitality. The seeds of *A. blitoides* and *A. graecizans* show a much lower catalase activity

than the seeds of *A. retroflexus*. They also show slower germination. It is not known whether the low catalase activity in these two species is due to a relatively low percentage of embryo material (the main seat of catalase) in the seeds, to some other specific difference in these seeds, or to lower vigor. The slow germination, however, may represent an unafter-ripened or dormant condition rather than low embryo vigor.

Seeds of *Amaranthus retroflexus* after-ripen during the first three or four months in dry storage. There is no evidence of embryo dormancy here. After-ripening is not marked by an increase in catalase activity. In this respect it resembles Johnson grass, but, unlike seeds of Johnson grass, there is no fall in catalase activity with age.

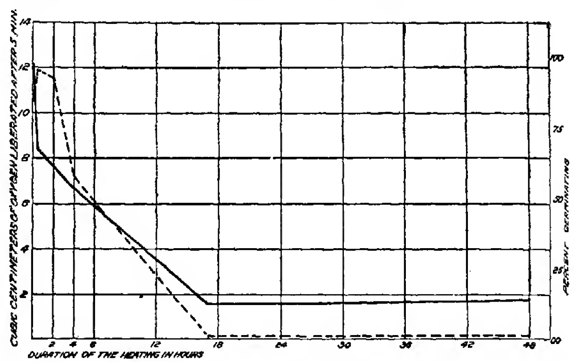

Fig. 3.—Graphs showing changes in viability and catalase activity in Johnson grass caused by heating air dry seeds at 81° C. for various lengths of time: Solid line=catalase; broken line=viability.

EFFECT OF HEATING AIR-DRY SEEDS UPON THEIR VITALITY, CATALASE ACTIVITY, AND RESPIRATION

Heating air-dry seeds causes a fall in their vitality as well as in their catalase activity, but the denaturing of the substances connected with viability and of the catalase do not parallel each other (Table XVIII).

Heating Johnson grass to 81° C. for half an hour to two hours reduces the catalase activity by a large percentage and improves the germination. Longer heating (four hours at 81° C.) causes considerable additional reduction in the catalase activity and a very decided fall in germination. Still longer heating (17 hours, at 81° C.) reduces the catalase to from 10 to 16 per cent of its original value and kills all the seeds. Heating to 100° C. for five hours kills all the seeds and destroys all their catalase. In the early stages of heat degeneration, as in time degeneration, the catalase falls faster than the viability; but some catalase activity persists after the seeds are all killed. The relative rate of degeneration of catalase and vitality with heating is shown in figure 3.

In heated seeds of Johnson grass the catalase activity more nearly parallels the respiratory intensity than it does the viability or vigor of the seeds, for seeds heated at 81° C. for one hour show a great lowering in the respiratory intensity, but an increase in the rate of germination. Tables XIX and XX show this relation. The respiration rate was determined by the method described by Grafe (*22, p. 357-361*), with the exception that Reiset instead of Pettenkofer tubes were used for absorbing the carbon dioxid. The intensity of respiration is initially more than twice as high in the unheated as in the heated seeds. It rises in both with time, but heated seeds gain on the unheated. The gain in intensity in both is due to the initiation of germination and the faster gain in the heated seeds is caused by the more rapid germination in them.

TABLE XVIII.—*Effect of heating air-dry seeds upon their catalase activity and vitality*

Seed used.	Amount of heating.	Oxygen liberated after— 1 min.	3 min.	5 min.	10 min.	Percentage of germination in 9 days.
		Cc.	*Cc.*	*Cc.*	*Cc.*	
Johnson grass 1 year old	None	4.4	9.3	12.7	17.8	82
Do	½ hour, 81° C	3.1	6.2	8.4	11.6	95
Do	2 hours, 81° C	2.9	5.7	7.7	10.3	92
Do	4 hours, 81° C	2.6	5.2	6.7	8.4	49
Do	17 hours, 81° C	.7	1.2	1.5	1.8	00
Do	48 hours, 81° C	.6	1.4	1.7	2.2	00
Do	5 hours, 100° C	0.0	0.0	0.0	0.0	00
Amaranthus retroflexus, 1917 crop.	None	7.5	12.5	14.5	16.9	100
Do	½ hour	.....	.....	.....	.....	25
Do	4 hours, 81° C	5.2	9.3	10.9	12.4	00
Do	17 hours, 81° C	4.7	7.1	9.3	10.7	00
Do	48 hours, 81° C	4.6	7.8	9.1	10.3	00
Do	4.5 hours, 100° C	.7	1.3	1.6	2.0	00

TABLE XIX.—*Effect of heating upon the respiratory intensity of 1-year-old Johnson grass seeds*

[Air-dry seeds heated 1 hour at 81° C., then soaked 24 hours at 20° C. Temperature of respiration chamber 20° C. Respiratory intensity=milligram of carbon dioxid produced by 10 gm., dry weight, of seeds per 24 hours]

Period.	Respiratory intensity. Heated.	Unheated.	Ratio, unheated to heated.
First (17 hours)	4.5	9.6	2.1
Second (22 hours)	12.9	13.8	1.1
Third (18 hours)	20.2	17.8	.86

TABLE XX.—*Effect of heating 1-year-old Johnson grass seeds for 1 hour at 81° C. upon rate of germination*

After various times at 25° C.	Percentage of germination.	
	Heated.	Unheated.
28 hours	12	8
47 hours	45	18

It is evident that moderate heating of Johnson grass seeds reduces both their catalase activity and respiratory intensity, while it increases the germinative capacity. It seems to lead to a more economic use of food—that is, a lower percentage of it is respired and a larger percentage is available for building new organs. A number of substances are capable of modifying the economic coefficient of plants (*25, p. 249–250*). This may be part of the benefit claimed for heating seeds, while more rapid germination is also a part. It would be interesting to know how long this effect persists in the seedlings from heated seeds. The fact that the ratio is less than 1 in the third period (Table XIX) does not indicate that the effect is lost. This low ratio is probably due to the more advanced stages in the germination of the heated seeds, for Rischawi (*33, p. 233*) has shown that respiratory intensity increases as germination progresses in the grasses.

Table XVIII shows that seeds of *Amaranthus retroflexus* lose their viability rapidly, while the catalase falls relatively slowly with heating at 81° C. The vitality is reduced to 25 per cent after 0.5 hour and to nil after 4 hours of heating, while the catalase activity is still about 60 per cent of its original intensity even after 48 hours' heating at 81° C. In seeds of *A. retroflexus* the catalase is comparatively heat-stable, while the substances essential to viability are comparatively heat-labile.

The findings of the last two sections may be summarized by saying that the catalase in air-dry seeds of *Amaranthus* spp. is comparatively heat- and time-stable; while the substances connected with viability are comparatively heat- and time-labile. In the main, exactly the reverse is true for Johnson grass.

EFFECT OF RETENTION IN A GERMINATOR

The catalase activity of Johnson grass is greatly reduced by retention in a germinator. This effect is shown in Table XXI. The seeds in a germinator at 20° C. for one year show from one-third to one-sixth the catalase activity of those dry-stored for the same period. This fall in catalase activity evidently begins soon after the seeds are placed in the germinator, for the seeds in the germinator for one month at 20° C. have less than one-half the catalase activity of the same seeds dry-stored. The fall in catalase activity is also much slower at 7° C.

TABLE XXI.—*Effect of retention in a germinator on catalase activity of seeds of Johnson grass*

Treatment of seeds.	Oxygen liberated after—			
	1 minute.	3 minutes.	5 minutes.	10 minutes.
	C.c.	*C.c.*	*C.c.*	*C.c.*
Dry-stored 1 year (1916 crop)	4.8	10.1	14.3	21.1
In germinator 1 year at 20° C.	1.5	2.3	2.7	3.4
Dry-stored 1 month (1917 crop)	7.2	15.7	21.5	31.3
In germinator 1 month at 20° C.	3.5	6.7	8.8	12.3
In germinator 1 month at 7° C.	4.3	9.4	13.1	18.6

This fall in catalase is accompanied by a fall in respiratory intensity. Table XXII shows the rate of respiration of two samples of the same collection of Johnson grass seeds, one stored dry and the other kept in a germinator at 20° C. for one year.

The gradual rise in the respiratory intensity of dry-stored seeds is due to the initiation of germination. At the close of the third period several had germinated in this lot, while none had germinated in the dormant lot.

TABLE XXII.—*Effect of retention in a germinator on the respiratory intensity of Johnson grass seeds*

[Intensity in milligrams of carbon dioxid per 10 grams, dry weight, per 24 hours at 20° C.]

Treatment of seeds.	Respiratory intensity.		
	First period.	Second period.	Third period.
1 year in germinator at 20° C.	3.6	3.4	3.1
1 year, stored dry	9.5	10.6	12.9

When freshly harvested Johnson grass seeds are put into a germinator at 20° C., they become more dormant. The senior writer (*15, p. 110-117*) has called this "secondary dormancy" and has mentioned that unfavorable germination conditions produce this in many seeds. If this deepened dormancy is generally accompanied by lowered respiration, it may have an important bearing upon the duration of dormancy of some seeds in nature. It is conceivable that imbibed dormant seeds in the ground may finally exhaust their stored foods by respiration and thus set a limit upon their longevity. Leaching may also play a part.

Reduction of the respiration, as in Johnson grass, will lengthen the period necessary for exhausting stored foods. If 75 per cent of the weight of the seed can be respired before death occurs, secondarily dormant Johnson grass seeds could lie in a germinator for 9.8 years at 20° C. before death would occur from exhaustion of stored foods. The period at 10° C. would likely be 2 to 3 times 9.8 years, in accord with the temperature quotient for respiration (*26, p. 153-154*). Without such a reduction in

respiratory intensity the possible longevity would be a little more than one-third as great, figured on the initial rate in the active seeds. Even if the longevity of imbibed seeds in the soil be dependent upon some contingent other than exhaustion of stored food, this reduction in respiration is of significance. It will leave more stored material for building purposes in case germination does occur after a considerable period in the soil.

Atwood (*3*) observed that unafter-ripened seeds of *Avena fatua* become more dormant after they have been in a germinator for a few days. The power of the seeds to absorb oxygen falls with the deepened dormancy. This may be parallel to the reduced respiration just discussed for Johnson grass. There is also the possibility, however, that oxygen absorption in the wild oats is limited by permeability characters and not by respiratory capacity. There is need of following the changes in catalase activity as well as carbon-dioxid production during the acquiring of deeper dormancy in this seed as well as many others.

It is quite within the range of possibility that longevity of imbibed seeds in the soil is commonly limited by the exhaustion of stored foods by respiration. Seeds of *Amaranthus retroflexus* retain their viability in the soil for at least 30 years (*18*) and those of *Brassica nigra* for many years (*28*). Both absorb a considerable percentage of water. The same is probably true of many other seeds. In such seeds respiration must be at a very low intensity to avoid death from food exhaustion.

When the air-dry seeds (carpel removed) of the peach are placed in a germinator the catalase activity rises continuously for more than 30 days and probably for more than 54 days, as shown by Table XXIII. The rate of rise in catalase activity is very much greater at 7° C. than at 20° or 25°, and it is somewhat greater at 20° than at 25°. The temperature 5° has been shown to be very nearly the optimum for after-ripening of the embryo of *Crataegus* spp., and it is an excellent temperature, if not the optimum, for the after-ripening of the peach embryo as well as other dormant embryos. The temperature 7° proved very favorable for the after-ripening of the peach, as after 54 days many of the seeds showed signs of germination, and 10 days later all had germinated, while all those in the germinator at 20° and 25° were dormant, except for the small percentage that germinated the first few days, as always occurs when carpel-free seeds are put into a germinator at 20° or 25°. These produce only stunted seedlings.

It would be of interest to know whether rise in respiratory capacity accompanies the very marked rise in catalase activity during the after-ripening of peach seeds. Greatly increased vigor of the seedlings resulting from after-ripened seeds as well as the rather general parallel found between catalase activity and respiration would suggest increased respiratory capacity.

TABLE XXIII.—*Effect of the temperature of the germinator on the increase in catalase activity of peach seeds; collected on September 15, 1917, dried, free from carpels, and put into the germinator on October 11, 1917*

Treatment of seeds.	Period.	Oxygen liberated after—			
		1 min.	3 min.	5 min.	10 min.
	Days.	*C c.*	*C c.*	*C c.*	*C c.*
Dry-stored	9	1.0	3.1	4.7	9.7
In germinator at 20° C.	9	3.4	9.6	13.2	20.4
In germinator at 7° C.	9	4.8	11.9	16.9	23.6
Dry-stored	30	.9	2.8	4.2	....
In germinator at 25° C.	30	4.8	11.7	16.0	21.9
In germinator at 20° C.	30	5.4	12.2	16.7	22.7
In germinator at 7° C.	30	13.4	29.3	36.2	....
Dry-stored	54	.9	2.6	4.1	....
In germinator at 25° C.	54	5.2	13.8	19.8	....
In germinator at 20° C.	54	5.1	15.3	23.0	....
In germinator at 7° C.	54	15.8	41.2	57.0	....

Certain contrasts between the behavior of peach and Johnson grass seeds are evident and very important.

Air-dry seeds of peach have very low catalase activity when compared with air-dry seeds of Johnson grass, Sudan grass, and other seeds of the same year's collection. This difference is magnified when it is recalled that the peach material is all from the embryo, the active part of the seed, while that of Johnson grass is only about 10 per cent embryo with about 90 per cent endosperm, material of low activity.

The peach seeds rise in catalase activity when being kept in the germinator, and the rise is much faster and greater at 7° than at 20° or 25° C. Johnson grass seeds fall in catalase activity with retention in the germinator, and the fall is more rapid at 20° than at 7°.

After-ripening in the peach involves fundamental time-requiring changes in the embryo. It progresses rapidly in a germinator at a low temperature, apparently not at all in dry storage, and very slowly, if at all, in a germinator at 20° C. or above. It is marked by a very great rise in catalase activity. After-ripening in the Johnson grass does not seem to involve fundamental time-requiring changes in the embryo. It proceeds well, if not best, in dry storage and is accompanied by a fall in catalase activity.

Other seeds (hawthorn and basswood) with dormant embryos behave like the peach in after-ripening. As in the peach, the catalase changes are accompanied by other chemical changes, already mentioned in the introduction.

RISE OF CATALASE ACTIVITY WITH GERMINATION

There is a big rise in the catalase activity of Sudan grass seeds with their germination. This is well shown in Table XXIV for the 1916 crop of Sudan grass. In this experiment the seeds were used without separating them into mature and immature lots. In the germinated

lot the seeds were grown at 20° C. until the coleoptiles were 4 to 6 cm. long. The germinated seeds were then allowed to dry for seven days in laboratory air before they were ground. As is seen from Table XXIV, the catalase activity is about doubled by germination to the stage reported above. Germinated Johnson grass seeds showed similar behavior. The reported activity for germinated seeds may be low, for, as has been shown, drying commonly decreases catalase activity in seeds. There seems no doubt that this rise in catalase activity is accompanied by a rise in respiratory intensity, for Rischawi (*33, p. 253*), has shown that when wheat grains germinate and grow in darkness at 21° C. the respiratory intensity rises from a value of about 14 the first day to 50 on the tenth day, where it is maintained until the sixteenth day. It gradually falls from there on, owing to the exhaustion of stored foods, until it attains a value of 15 on the twenty-sixth day.

TABLE XXIV.—*Catalase activity of germinated and ungerminated Sudan grass seeds*

Condition of seed.	Oxygen liberated after—			
	1 min.	3 min.	5 min.	10 min.
	Cc.	*Cc.*	*Cc.*	*Cc.*
Dry-stored	9.4	21.4	29.0	38.4
Germinated, coleoptile 4–6 cm	19.8	42.8	58.0	76.4

SOLUBILITY OF THE CATALASE OF SEED

Loew (*30*) found that the catalase of various plants consisted of two sorts: insoluble, or α-catalase, and soluble, or β-catalase. The relative proportion of these two constituents varied greatly in different plants as well as in different organs of the same plant. It was thought well to see whether there is any correlation between the relative time and heat stabilities of the catalases of amaranthus and Johnson grass seeds and the proportions of insoluble and soluble catalases in them. The data on this point are reported in Table XXV. In these experiments either 0.2 gm. of seed powder suspended in 10 cc. of water or the filtered extract of 0.2 gm. of seed powder in 10 cc. of water was used for each determination. Ten cc. of dioxogen were added to this, and the volume of oxygen liberated was measured. An excess of calcium carbonate ($CaCO_3$) was kept in contact with the materials at all stages of the process to protect against injury by acids.

Four different treatments were used for the materials of each sort of seeds: (1) Powder added to the water just before the determination and the whole suspension used in the determination; (2) powder suspended in the water and shaken for one hour at 25° C. and the whole suspension used in the run; (3) powder treated as the last but only the filtrate passing through a C. S. & S. 595 filter paper used in the deter-

mination; (4) same as the last but only the filtrate passing through a porous clay (fine Berkefeld) filter used in the run.

TABLE XXV.—*Solubility of catalases of amaranthus and Johnson grass seeds*

Seed and treatment.	Oxygen liberated after— 5 min.	10 min.
Amaranthus retroflexus:	*Cc.*	*Cc.*
Powder	19.0	23.6
Powder shaken with water 1 hour at 25° C.	15.5	19.1
Filtered extract (C. S. & S. filter No. 595)	9.0	11.0
Filtered extract (Berkefeld)	8.5	10.1
Johnson grass:		
Powder	11.0	16.2
Powder shaken with water 1 hour at 25° C.	10.8	15.9
Filtered extract (C. S. & S. filter 595)	3.2	5.1
Filtered extract (Berkefeld)	1.5	2.1

In amaranthus shaking the powder with water for one hour reduced the catalase noticeably. The catalase of this seed seems to be sensitive to such agitation. Filtering through either the filter paper or the Berkefeld filter reduced the activity somewhat more than 50 per cent, the latter showing slightly the greater reduction. In the Johnson grass shaking for one hour with water gave scarcely any reduction in activity, while filtering through the filter paper gave a reduction of about 70 per cent, and filtering through a Berkefeld filter a reduction of about 86 per cent.

On this basis somewhat more than 50 per cent of the catalase of amaranthus seeds is insoluble, while 70 per cent or more of that of Johnson grass seeds is insoluble. This, however, takes no account of the portion of catalase that may be chemically united with or adsorbed by the insoluble seed powder and the filters. It is likely also that the catalase complex is in the colloidal state and that the portion in the lower degrees of dispersion is held back by the fine filter, especially after it is blocked by colloidal materials. The proportion of soluble and insoluble catalase in these two seeds is not such as to throw any light on the relative time and heat stability of the catalases of them.

OXIDASE IN SEEDS

All oxidase determinations were made at 30° C. in the Bunzell simplified apparatus (*10*) either with or without caustic boats suspended from the manometer for absorption of carbon dioxid. All the material used was ground so that it would pass through bolting cloth with 70 or 80 meshes per inch. Seeds of Johnson grass, Sudan grass, or Tunis grass-sorghum hybrid, when used without removing the scales, were ground until about 85 per cent by weight pass through the bolting cloth, the remaining 15 per cent being discarded.

On account of its relative inactivity, the quantity of material used was necessarily large, usually 250 or 500 mgm. for each determination. The quantity of reagents used was uniform in all determinations, 10 mgm. of a solid reagent or 2 drops of para-cresol for each determination.

The different experiments were run for a length of time varying from 4½ to 24 hours.

OXIDASE ACTIVITY TOWARD DIFFERENT REAGENTS

Ten different lots of seeds, comprising Johnson grass, *Amaranthus retroflexus*, Sudan grass, wheat, and a fixed Tunis grass sorghum hybrid were tested for oxidase activity with pyrogallol, pyrocatechol, and para-cresol.[1] With very few exceptions pyrogallol gave the greatest activity, para-cresol next, and pyrocatechol least (*11*). Frequently the oxidase reaction toward pyrogallol and pyrocatechol proceeded at practically uniform rates throughout the experiment even when the experiment was run for nearly 24 hours. In some of the experiments, however, the rate slowed down perceptibly after the first few hours, although it never reached a definite end point with any of the reagents used. This is in marked contrast to the course of oxidase activity shown by Bunzell (*9*) with a large variety of plant materials. In his work the reaction seemed to be practically complete in 2 or 3 hours.

The activity towards para-cresol usually started very slowly (probably on account of the limited solubility of the reagent), increased somewhat in rate after the first few hours so that the total reduction in pressure sometimes temporarily exceeded the total reduction with pyrogallol as the reagent, and then decreased in rate so that the pressure reduction again became considerably less than with pyrogallol.

All of the material used had relatively low activity. The greatest activity of the most active material used (except scales and sterile florets) caused a total reduction of pressure of about 95 mm. in 19 hours with 250 mgm. of the material and 17 cc. as the active volume of gas. This is equivalent to the absorption of only about 10.7 cc., or 15 mgm. of oxygen in 24 hours per gram of the ground seed material; yet this is more than 10 times as great an activity as that shown by ground peach embryos. Johnson grass seeds were more active than any other seeds used, with amaranthus a close second. The Tunis grass-sorghum hybrid was somewhat less active, and Sudan grass (1911 seed) very much less active. Wheat (entire caryopses) showed very little oxidase activity, and peach embryos practically none at all.

DETAILED ACCOUNT OF OXIDASE EXPERIMENTS

The results given in the following paragraphs were obtained with pyrogallol as the reagent. The amount of oxidase material used was not

[1] Preliminary trials with Johnson grass seeds showed practically no activity toward hydroquinone and tyrosine.

uniform in the different experiments. When different amounts of the same material were used in duplicate determinations the smaller quantity invariably showed greater relative activity than the larger quantity. On account of the lack of proportionality between the amount of seed material used and intensity of oxidase activity indicated, the latter is reported in the following paragraphs in terms of reduction of pressure in a given time, with a given amount of seed material.

OXIDASE ACTIVITY IN EMBRYO AND ENDOSPERM

The material used was Stoner wheat, harvested in July, 1917, and well-matured Sudan grass seed about 6 years old, the entire caryopses ground whole and the endosperm and embryo ends ground separately being used. For this purpose the Sudan grass caryopses were simply cut in two just distal to the embryos. The wheat kernels were cut diagonally so as to include only a small amount of endosperm with the embryo. The embryo ends thus cut off constituted 11.3 per cent of the entire caryopses. The embryo ends were ground until 8.7 per cent by weight of the entire caryopses passed through the bolting cloth, the residue of endosperm and bran being discarded. The remaining 88.7 per cent of the caryopses was ground until 34.7 per cent of the entire caryopses, consisting of the inner portion of the endosperm, passed through the bolting cloth; the remainder was discarded.

Table XXVI shows the oxidase activity of endosperm and embryo ends compared with that of the entire caryopses. The embryo ends showed very much greater activity than the endosperm ends, while the activity of the whole caryopses was intermediate.

TABLE XXVI.—*Oxidase activity of embryo and endosperm ends of caryopses compared with that of the entire caryopses*

Material.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, embryo to endosperm.
Stoner wheat:	*Mgm.*		
Whole caryopses	500	9.5 mm. in 4½ hours	
		55.5 mm. in 19½ hours	
Embryo ends [a]	500	27 mm. in 5 hours	54
Do.	250	17.5 mm. in 5 hours	
Endosperm ends [b]	500	0.5 mm. in 5 hours	
Sudan grass seed (1911?):			
Whole caryopses	400	15.5 mm. in 7 hours	
Embryo ends	500	22 mm. in 6 hours	5.5
Endosperm ends	500	4 mm. in 6 hours	

[a] 8.7 per cent, by weight, of the grain, ground from 11.3 per cent portions were used. The coarse residue was discarded.

[b] 34.7 per cent, by weight, of the grain, ground from 88.7 per cent portions were used. The coarse residue was discarded.

OXIDASE ACTIVITY AS RELATED TO MATURITY OF SEED

The material used was Sudan grass seed about 6 years old and Johnson grass seed about one month after harvesting. The separation of the

large, heavy, thoroughly mature seeds from the lighter, more poorly matured seeds was made by means of vertical air-blast seed separator. The results of the experiment are given in Table XXVII.

TABLE XXVII.—*Oxidase activity of mature and immature seeds*

Material and condition.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, immature to mature.
Sudan grass, whole caryopses:	*Mgm.*		
Mature	400	13.5 mm. in 7 hours	0.84
Immature	400	13 mm. in 7 hours	
Sudan grass, embryo end:			
Mature	500	22 mm. in 6 hours	
Immature	400	15.5 mm. in 6 hours	
Johnson grass, caryopses in scales:			
Mature	500	35 mm. in 6 hours	1.30
Do	500	45.5 mm. in 6 hours	
Immature	400	42 mm. in 6 hours	

The mature Sudan grass seed was slightly more active than the immature, whether the whole caryopses or only the embryo ends were used. With Johnson grass seed, however, the immature seed was considerably more active than the mature seed.

OXIDASE ACTIVITY AS RELATED TO AGE OF SEED

The material consisted of a sample of Johnson grass seed about 3 years old; two samples of Johnson grass seed from a common original selection, one of which was 1 year old and the other about 1 month old; and two samples of seed of *Amaranthus retroflexus*, one over 2 years old and the other about 2 weeks after harvesting. The results of the experiments are given in Table XXVIII.

TABLE XXVIII.—*Oxidase activity as related to the age of the seed*

Material.	Age.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, old to new.
		Mgm.		
Johnson grass 1417	3 years	500	10.5 mm. in 4½ hours	0.35
Johnson grass 8599	1 year	500	29.5 mm. in 4½ hours	
Do	do	250	68 mm. in 19 hours	.72
Do	3 weeks	250	95 mm. in 19 hours	
Amaranthus retroflexus	2 years	500	19.5 mm. in 4½ hours	.51
Do	2 weeks	500	38.5 mm. in 4½ hours	
Do	2 years	250	25.5 mm. in 10 hours	.57
Do	2 weeks	250	45 mm. in 10 hours	

The intensity of oxidase activity decreases markedly with age. One-year-old Johnson grass seed was a little more than two-thirds as active

as fresh seed; two-year-old amaranthus seed was about one-half as active as fresh seed; and three-year-old Johnson grass seed was only about one-third as active as one-year-old seed.

OXIDASE ACTIVITY AS RELATED TO AFTER-RIPENING AND CONDITION OF STORAGE OF SEED

The oxidase activity of samples of Johnson grass seed a little over a year old, which had been stored dry in cloth sacks at room temperature, was compared with that of portions of the same original samples which had been stored for a year between moist blotting papers at 20° C.[1] Comparative tests were made also with peach embryos which had been stored for 1 month air-dry at room temperature and in moist blotting paper at 7° C.,[2] and similarly at 25°. The peach seeds were taken from the stony carpels before the period of storage, and the coats were removed from the embryos just before grinding the latter for oxidase determinations.

Table XXIX gives the results of the experiments. One-year-old Johnson grass seed which had been kept at a temperature slightly below the minimum for germination, though otherwise under germination conditions, showed considerably less oxidase activity than seeds from the same original lot which had been stored dry. After grinding both lots were dried in a desiccator before weighing. Peach embryos showed very little oxidase activity under any conditions, and this activity did not change significantly during incubation even at the after-ripening temperature, 7° C.

TABLE XXIX.—*Oxidase activity as related to after-ripening and condition of storage of seed*

Material and condition.	Quantity of powder used.	Reduction of pressure.	Ratio of activity, wet to dry.
Johnson grass 8599 (collected on September 9, 1916):	*Mgm.*		
Stored dry 1 year at room temperature.	500	29.5 mm. in 4½ hours	....
Stored in germinator 1 year at 20° C.	250	13.5 mm. in 4½ hours	....
Johnson grass 8599 (collected on September 23, 1916):			
Stored dry 1 year at room temperature.	250	24.5 mm. in 6½ hours	0.80
Stored in germinator 1 year at 20°C.	250	19.5 mm. in 6½ hours	
Peach embryos:			
Stored dry at room temperature	500	6.5 mm. in 24 hours	....
Incubated 45 days at 7° C.	500	3.5 mm. in 24 hours	....
Incubated 45 days at 25° C.	500	3.5 mm. in 24 hours	....
Stored dry at room temperature	250	9 mm. in 1½ 9 hours	....
Incubated 42 days at 7° C.	250	9 mm. in 1½ 9 hours	....

[1] Johnson grass seeds were kept in a condition of secondary dormancy by storing in moist blotters at 20°.
[2] Peach embryos stored thus after-ripen rapidly at 7°, but not at 25°.

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Supplementary experiments with peach embryos, with pyrocatechol and para-cresol as oxidase reagents, showed practically no oxidase activity either with dry-stored embryos or with after-ripened embryos. In this connection the color reaction in the oxidase reagents is of interest. The pyrocatechol and pyrogallol solutions became very slightly colored during the experiments. This coloring was barely perceptible with the material which had not after-ripened and slightly more intense with after-ripened material. With para-cresol as the reagent and in control lots with no reagent there was no change in color.

OXIDASE ACTIVITY AS RELATED TO GERMINATION

Johnson grass seeds collected on September 9, 1916, were incubated at 20° C. for several days late in November, 1917, and then with the temperature alternation 25° to 40° until nearly all had germinated. Seeds which showed no sign of germination were then picked out, ground, and tested for oxidase activity in comparison with dry seeds and with seeds which had germinated, and in comparison also with the sprouts produced by another sample of the same original lot which had been germinated at 20° after first heating for 1 hour at 81° and sterilizing with 5 per cent silver nitrate.[1]

Many of the seeds in the last-mentioned sample had reached an advanced stage in germination, some coleoptiles being over 10 cm. long. The elongated coleoptiles and a few of the roots were broken off, crushed, dried before a fan, and ground for oxidase determinations.

The germinated seeds which had been ground entire were in various stages of growth. The longest coleoptile was 8 cm. long and the longest root 4 cm., but a large majority were less than one-half as long as these. Before being ground these germinated seeds had been crushed and dried before a fan. Table XXX gives the results of these experiments.

TABLE XXX.—*Relation of oxidase activity to germination*

Material and condition.	Quantity of powder used.	Reduction of pressure.
Johnson grass 8604 (collected on Sept. 9, 1916):	*Mgm.*	
Ground dry	333	20.5 mm. in 7½ hours.
Incubated several days at 20° C., then at 25° to 40° C.—		
Not germinated	333	16.5 mm. in 7½ hours.
Germinated	333	16.0 mm. in 7½ hours.
Sprouts	167	14.0 mm. in 7½ hours.

[1] Although the sterilized seeds were very thoroughly washed, first with distilled water, then with sodium chlorid solution, and finally with distilled water, oxidase activity in the ground seeds was almost completely inhibited, probably by adsorbed silver. Of course the sprouts were free from this inhibiting agent.

The dry seeds apparently showed somewhat greater oxidase activity than the imbibed seeds, either germinated or not germinated. The differences, however, are probably only apparent, as the ground imbibed seeds at the time of weighing contained a higher percentage of moisture than the ground dry seeds. If one assumed a moisture content of 8 per cent for the powder from the dry seeds and a moisture content of 26 per cent for the powder from the imbibed seeds, the intensity of activity would be identical when calculated to a dry-weight basis.

The ground sprouts were considerably more active than the whole seeds, but the ratio is no greater than would be expected from the comparison of embryo ends with caryopses (see Table XXVI); therefore no increase in activity upon germination is indicated.

OXIDASE ACTIVITY OF NONLIVING STRUCTURES

A very interesting fact is the relatively high oxidase activity of nonliving structures in which catalase activity is very slight and respiration presumably absent—viz, the bracts or scales which inclose the mature caryopses of Sudan grass and Johnson grass and the dry abortive or sterile florets of Johnson grass.[1] Johnson grass and amaranthus seeds showed greater activity than the other kinds of seeds. It was at first thought that this fact might be related to the intense pigmentation of the scales in one case and of the pericarps in the other. As Table XXXI shows, comparative experiments with scales and caryopses proved the contrary.

TABLE XXXI.—*Oxidase activity of nonliving structures*

Material and condition.	Quantity of powder used.	Reduction of pressure.
Johnson grass 8599 (collected on Sept. 22, 1917), medium ripe:	*Mgm.*	
Caryopses	500	26 mm. in 5 hours.
Scales (medium colored)	250	24 mm. in 5 hours.
Johnson grass 8599 (collected on Sept. 9, 1916), very ripe:		
Caryopses (removed from scales by grinding in coffee mill, fall of 1916).	500	15 mm. in 5 hours.
Scales (black)	500	Do.
Sterile florets	400	20. 5 mm. in 1 hour. 31. 0 mm. in 2 hours. 49. 0 mm. in 5 hours.
Sudan grass, mature:		
Endosperm ends of caryopses	500	4 mm. in 6 hours.
Embryo ends of caryopses	500	7. 5 mm. in 2¼ hours. 22. 0 mm. in 6 hours.
Scales, light straw-colored	500	15. 5 mm. in 1 hour. 21. 5 mm. in 2⅛ hours. 33. 0 mm. in 6 hours.
Scales, control, no reagent	500	6 mm. in 2⅛ hours. 10 mm. in 6 hours.

[1] Each fertile floret in Johnson grass is accompanied by a sterile floret which never develops a caryopsis.

The scales of only moderately well matured Johnson grass seed showed a somewhat greater oxidase activity than the caryopses, but with unusually well matured 1-year-old seed having intensely pigmented scales the activity of the scales was exactly the same as that of the caryopses. At the same time the sterile florets, though functionless structures scarcely pigmented at all, in which all vital activities must have ceased at a time corresponding to an early stage in the development of the caryopses in the accompanying fertile florets, were about four times as active as either scales or caryopses. In fact, these sterile florets showed greater oxidase activity in limited time (5 hours) than any other material studied in the investigation.

The light, straw-colored scales of Sudan grass showed 50 per cent greater total oxidase activity in 6 hours than the embryo ends of the caryopses, and about 8 times as great as the endosperm ends.

A very noticeable feature of the high oxidase activity of Sudan grass scales and sterile florets of Johnson grass is the high initial rate of activity followed by a rather rapid and progressive decline in rate, though activity had not ceased when the experiments were concluded. As shown in Table XXXI, the decrease in pressure with this material was nearly half as great at the end of 1 hour as at the end of 5 or 6 hours. This is in marked contrast to the progress of the reaction with the other kinds of material used in the investigation, and suggests rather the type of reaction reported by Bunzell. By the end of the second hour the initially high rate of activity of Sudan grass scales fell below the constant rate maintained by the embryo ends of the caryopses.

It is interesting to notice that there was a similar reaction with decrease in rate after the second hour in a control with ground scales and no reagent. In this control tube the reduction of pressure was 6 mm. in $2\frac{1}{6}$ hours, and 10 mm. in 6 hours. With most of the other kinds of material used, the controls showed little or no change in pressure until fermentation began, after which the reduction in pressure was frequently rapid. No control was run with the sterile florets.

One might perhaps see a correlation between the high oxidase activity of these structures (scales and sterile florets) and the high oxidase activities caused by agencies which retard normal growth, as reported by Bunzell (9). He suggests a general relation between retardation of growth, from any cause whatever, and rise in oxidase activity. In the case of the sterile florets retardation or suspension of function has proceeded so far as entirely to prevent the formation of seeds and to withdraw all vitality from the florets.

EFFECT OF MERCURIC CHLORID UPON OXIDASE ACTIVITY

Previous work has shown that mercuric chlorid ($HgCl_2$) has a strong forcing action upon the caryopses of Johnson grass, the maximum effect being obtained with a *M/2,000* solution. Table XXXII shows the

oxidase activity of 250-mgm. samples of fresh seed of Tunis grass-sorghum hybrid and of Johnson grass seed in secondary dormancy with *M/2,000* mercuric chlorid replacing distilled water in the oxidase baskets. The results with distilled water are also given.

The mercuric-chlorid solution had only a slight depressing effect upon oxidase activity. Either this oxidase is very much more resistant to mercury poisoning than some other enzyms or else the concentration of the solution was very greatly reduced by adsorption to the large amount of powder used. McGuigan (*31*) found the activity of diastase completely inhibited by *M/30,000* mercuric chlorid. Caldwell (*14*) showed that the activity of bromelin was completely inhibited by *M/45,000* to *M/75,000* mercurous nitrate ($HgNO_3$) and mercuric nitrate $Hg(NO_3)_2$.

TABLE XXXII.—*Effect of M/2,000 mercuric chlorid upon oxidase activity*

Material.	Reagent.	Reduction of pressure.
Tunis grass-sorghum hybrid (collected on Sept. 14, 1917.)	Pyrogallol + *M/2,000* mercuric chlorid.	16 mm. in 6 hours.
Do.	Pyrogallol in distilled water.	20 mm. in 6 hours.
Johnson grass 8599 (collected on Sept. 23, 1916), incubated at 20° C.	Pyrogallol + *M/2,000* mercuric chlorid.	12.5 mm. in 6 hours.
Do.	Pyrogallol in distilled water.	14 mm. in 6 hours.

SUMMARY

(1) Measurement of the oxygen liberated from hydrogen peroxid by the addition of an excess of powdered seeds (plant catalase) provides a convenient method of determining the concentration of the hydrogen peroxid used.

(2) In the determination of the catalase of seeds it is necessary to neutralize the hydrogen peroxids used, for the acidity in all commercial hydrogen peroxids tried was sufficient to reduce greatly the catalase activity. The seeds studied bear no inhibiting acids, but show buffer action against the acids of the hydrogen peroxids.

(3) Excessive pulverization of seeds reduces their catalase activity. Powder of Johnson grass gave maximum activity when passed through a 70-to-80-mesh bolting cloth, and crimson clover when passed through a 100-mesh.

(4) In powdered seed material (Johnson grass) stored in a desiccator at room temperature the catalase degenerates rather rapidly, losing 70 per cent of its activity in 54 days. Morphological integrity insures much slower degeneration.

(5) In the embryo of wheat the catalase activity is 28 to 29 times that of the endosperm. Burlakow (*4*) found the respiratory activity of the

embryo 20 times that of the endosperm. In other grasses studied the catalase activity of the embryo was many times that of the endosperm. The oxidase activity is likewise much higher in the embryo than in the endosperm.

(6) The physiologically inactive organs (sterile florets and caryopsis scales) of grass seeds show only a small fraction of the catalase activity shown by the caryopses. This likely agrees with the respiratory intensity. The oxidase is as active, or in some cases several times as active, in the nonliving as in the living organs.

(7) Equal weights of immature caryopses of Johnson grass or Sudan grass and of seeds of *Amaranthus retroflexus* give much greater catalase activity than mature ones. The activity of an equal number of the caryopses, mature and immature, of the two grasses is about equal. The oxidase activity on weight basis in Sudan grass is slightly higher in mature seeds than in immature, and the reverse holds for Johnson grass.

(8) Drying the seeds that have been in a germinator reduces enormously the catalase activity in the peach, noticeably reduces it in Johnson grass, but not at all in basswood.

(9) The catalase in air-dry Johnson grass seeds is comparatively time- and heat-labile, while that in air-dry amaranthus seeds is relatively time- and heat- stable. The respiratory intensity (measured after the seeds are imbibed) parallels the catalase activity in the first species. There is nothing to indicate such a relation in the latter. The difference in time and heat stability is not determined by the relative proportions of soluble and insoluble catalases in the two seeds. The nonliving organs of Johnson grass (sterile florets and caryopses scales) show much faster time degeneration of their catalase than the living embryo.

(10) Retention in a germinator, not furnishing conditions for germination, greatly reduces the catalase activity of Johnson grass seeds. One year at 20° C. reduces it more than 66 per cent, and 1 month more than 50 per cent below that of the same crop dry stored. The rate of fall in the catalase activity is decreased by lowering the temperature of the bath. Retention in a germinator affected the oxidase activity in the same direction but to a much less degree.

(11) The fall in catalase activity mentioned in the preceding paragraph is accompanied by a commensurate fall in respiratory intensity. A similar response seems to occur in *Avena fatua*, and probably occurs in many other seeds. This reduction in respiratory intensity is of great significance in conserving stored foods in seeds lying in the ground, dormant and imbibed, for many years.

(12) The catalase activity in dry peach seeds is very low, but rises as the seeds lie in the germinator imbibed. The rise continues for weeks and is much more rapid at 7° C. than at 20° or 25°. The optimum temperature for after-ripening seems to be optimum for catalase increase. In other seeds having dormant embryos, so far as studied by other investi-

gators, the same relation holds. In the peach the oxidase activity (Bunzell method) decreases with after-ripening but autocoloration of the ground seed mass exposed to the air increases.

(13) In amount of catalase and in the general behavior of their catalases Johnson grass and Sudan grass seeds are very similar and one finds here no explanation for their marked difference in dormancy and requirement of alternate temperatures for germination. The same is true of the oxidases of the two seeds, so far as our studies have gone.

(14) The catalase activity of grass seeds rises rapidly as their germination progresses. This parallels the rise in respiratory intensity. There is no rise in oxidase activity with germination.

(15) In Johnson grass seeds there seems to be a close correlation between catalase activity and respiratory intensity (factors that modify one modify the other similarly), but there is not a very close correlation between either of them and the vitality of the seeds or vigor of the resulting seedling. In these seeds catalase determination proved an excellent quick method of estimating respiratory intensity and led to the discovery of several interesting features in their respiration. In these seeds the catalase also decreases with age and it is a fair measure of age in continuously dry-stored seeds.

(16) In amaranthus seeds there is no evidence of a correlation between catalase activity on one hand and respiratory intensity, vitality, or age on the other. This lack of correlation may be connected with the greater time and heat stability of the catalase of amaranthus.

(17) So far as studied to date, seeds that after-ripen with dry storage but which do not have embryos with dormancy self-imposed at any time either show no change in the catalase activity (amaranthus) or a decrease in it (Johnson grass) with after-ripening.

(18) Seeds that after-ripen in a germinator at low temperature (commercial layering) and in which the dormancy of the embryo is self-imposed and the embryo experiences fundamental time-requiring changes for after-ripening, show a great increase in catalase activity with after-ripening (hawthorns, basswood, peach).

(19) It has been suggested that the gradual loss of vitality in dry-stored seeds with age is due to the time denaturing or time coagulation of embryo proteins. If this be true, it is evident that the time denaturing of the embryo proteins essential to vitality and the time denaturing of catalase are quite distinct, for in no old seeds studied is there a close parallel between catalase activity and vitality.

(20) It is evident from the great variations in catalase behavior in the several seeds studied that one can not draw general conclusions for the catalase behavior in all seeds, but it seems evident from the data in this paper that seeds will fall into several physiological types for each of which more or less general conclusions can be drawn.

(21) Catalase activity of seeds seems to parallel physiological behavior much more generally than does oxidase activity.

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THE MEADOW PLANT BUG, MIRIS DOLABRATUS[1]

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INTRODUCTION

The meadow plant bug, *Miris dolabratus* L., presents a number of interesting problems, biologic as well as economic, and, considering its great abundance over a large area of the eastern United States and Canada during the past 40 years, it seems strange that it has not received more careful investigation.

My own attention was attracted by its appearance in immense numbers in northern Ohio at about the time of my removal to that State in 1898. It was entirely unknown to me from previous field collecting, and specimens I had seen had been collected in western New York by Mr. E. P. Van Duzee about the year 1888.

My attention was again forcibly attracted to the species by its great abundance in Maine in 1914, when I encountered it while studying the meadow leafhoppers. Reference to the literature indicated the almost total neglect of the species in this country, and almost nothing concerning its economic importance was found. It seemed, therefore, well worth a special study, and I was gratified to be able to arrange with the Maine Agricultural Experiment Station to undertake a summer's study of the species at Orono.

As an Old World species the insect has evidently been familiar since it was described by Linnæus (*1758, p. 449*),[3] and has had frequent mention by later writers, who have treated it simply from the systematic standpoint. Wolff (*1802, p. 115–116, fig. 109–110*) indeed gives a recognizable figure of the nymph in one of the later instars, also a rough sketch of the egg; but, so far as noted, no detailed study of the life history, habits, or economic status has been made, even in the regions where it has been longest known.

DISTRIBUTION OF THE SPECIES

The range of the species is evidently throughout the Palearctic region, as the European records cover the territory to the Mediterranean, and the Asiatic seem to include all north of the Himalaya Mountains at least.

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[1] Papers from the Maine Agricultural Experiment Station: Entomology 99. Contribution from the Department of Zoology and Entomology, Ohio State University, No. 53.

[2] I am indebted to a number of persons for assistance in the preparation of this report, especially to Dr. Edith M. Patch, of the Maine Experiment Station, for facilities to carry forward the study and to Mr. R. K. Fletcher for careful attention to field observations and to the entomologists of various States who have kindly taken the trouble to send records for their territory.

[3] Bibliographic citations in parentheses refer to "Literature cited," p. 199–200.

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Oshanin (*1909, p. 779*) lists the distribution as—

Scandinavia, Batavia, Britannia, Belgica, Germania, Helvetia, Gallia, Lusitania, Hispania, Moldavia, Serbia, Romania, Hungaria, Rossia fere tota, Caucasus, Sibiria. Regio nearctica (Canada, eastern United States).

PUBLISHED RECORDS OF OCCURRENCE IN AMERICA

The first record of the species in America that I can identify as referring to its occurrence in America is the one by Uhler (*1878, p. 397*). Provancher (*1872, p. 78*) recorded it for the vicinity of Quebec when he listed it as a new species under the name "*Miris belangeri*" in 1872 and later (*1886, p. 104*) referring it to the European species under the name "*Leptoterna dolabrata* L." In Uhler's Check List of Hemiptera Heteroptera (*1886, p. 17*) it appears under the name "*Leptoterna dolobrata*" with locality as "E. St." Van Duzee (*1887, p. 70*) says:

May to August. In dry fields. Probably our most abundant Hemipter. It attains full development about June 1, and frequently appears in immense swarms in favorable localities.

Later (*1894, p. 176*) he says:

Often appears in immense swarms toward the last of June on grass in hayfields and pastures.

Van Duzee (*1905, p. 550*) also records the species for the Adirondack Mountains and (*1908, p. 111*) for Quinze Lake, Province of Quebec, Canada, in 1907. Slosson (*1894, p. 5*) records the species for Mount Washington, New Hampshire, above the 5,500-foot altitude.

Webster and Mally (*1897, p. 41*) barely mention the species as abundant on the heads of timothy in 1896, which is the first published record for Ohio, though Mr. Hart says a specimen is in the Illinois collection sent from Columbus by Prof. C. M. Weed, presumably about 1888 or 1890.

The species is recorded for New Jersey for a number of localities by Smith (*1900, p. 128; 1910*), but with no definite dates. It is also listed by me (*1900, p. 76*) in the Ohio list, and its abundance in Maine is referred to by Patch (*1908*, (*p. 363*) and by me (*1916, p. 56*).

Finally a record of the rearing of a parasite, *Phorantha occidentis*, by Leonard (*1916*) indicates its occurrence in 1915 in New York.

None of these records, except Uhler's, raises the question of the derivation of the species, but from the facts that there were no early records of damage in this country and that there seemed to be a distinctly westward dispersal the possibility of its being an introduced species associated with timothy (*Phleum pratense*) as its principal food plant seemed to warrant an effort to determine this point.

The following letter with note concerning the species was sent to a number of entomologists in the various States and to Dr. C. Gordon Hewitt, Dominion Entomologist, of Canada.

ORONO, MAINE, *July 5, 1916.*

DEAR SIR: The writer is engaged upon a study of a meadow Capsid (*Miris dolabratus*) for the Maine Agricultural Experiment Station, and since the species is abundant over a considerable area of the northeastern United States it is desirable to secure data from as large an area as possible. With the cordial approval of the Station authorities and in the hope that the results may have more than local value I am asking assistance in securing such data from the entomologists in a number of adjacent states. The data desired covers such items as occurrence, abundance, recognized injury, grasses affected and any matter bearing on the life history and habits in the localities reported. Any such information will be welcomed and duly credited.

The species is one of the larger Capsids, elongate in form, yellow or sometimes reddish with dark markings and is found usually in great numbers on heads of timothy or other meadow grasses in midsummer. It is a common species in the Palearctic region and has been known in America for about forty years, the first record apparently being by Provancher for the vicinity of Quebec. There are several facts which point to the possibility that it may have been introduced from Europe somewhat recently and any data as to time of first appearance in any locality will be especially helpful in determining rate of dispersal. If the species is not positively known I will be glad to receive and identify specimens that may be suspected.

With sincere thanks for any information either as to presence or absence in your locality,

Very truly yours,

HERBERT OSBORN,
Experiment Station, Orono, Maine.

Prof. H. T. Fernald, of the Massachusetts Experiment Station, replied as follows:

My first note on this insect shows that I took it June 23, 1882, at Orono. I may have taken it before this, but have no note on it. I have also a note that larvæ of it were very abundant June 15th, 1883, at Orono. My remembrance of it is that it was always very abundant as far back as 1880 at least, when my observations began.

Here at Amherst it has been abundant ever since our collections were made, so far as I can learn. We get it sweeping over our grass fields, but as these fields are nearly always more or less mixed grasses, I have not been able to determine exactly which kind of grass it attacks, and in fact I have not given much attention to this insect. Here we get the larvæ in abundance about the first of May, varying with the season. Adults begin to appear early in June. I regret to say that I have no further data on this subject, but am confident that a careful examination would show a second generation here the same season at least. Whether there is a third, I do not know.

Prof. W. E. Britton, of Connecticut, wrote:

I have your letter of July 5th and wish to inform you that *Miris dolabratus* Linn. is very common in Connecticut, in fact, so common that in sweeping we do not save the specimens. I have never made a study of this species and can not tell you offhand just what species of grass it attacks.

The following records are given for Connecticut localities:

New Haven, June 16, 24, 1902 (E. J. S. Moore), June 8, 1904 (W. E. Britton); Brooksvale, Sheshire, July 30, 1902 (W. E. Britton); Branford, June 27, 1904 (H. L. Vierick); Mount Carmel (Hamden), June 10, 1908 (W. E. Britton); Stratford, June 29, 1908 (W. E. Britton); Stonington, June 14, 1906 (W. E. Britton); Wallingford, June 15, 29, 1912 (D. J. Caffery); Wetherfield, June 24, 1913 (L. B. Ripley).

Dr. E. P. Felt, State entomologist of New York, wrote as follows:

Replying to yours of the 5th instant would state that *Miris dolabratus* is a rather common and widely distributed insect in this State, attaining maturity about the middle of June. Occasionally it is exceedingly abundant, as was the case June 14, 1898, at Trenton Falls, where it literally swarmed in a field of timothy. You doubtless appreciate that unless such insects are extremely numerous and kill or nearly destroy the grass comparatively little attention is paid to them.

The following records, supplied by Dr. Felt, are from the State Museum of New York:

Albany, June 19, 1900, June 13, 1903, June 25, 1901, June 17, 19, 1899; Chazy Lake, June 28, 1913; Crane Pond, July 2, 1897; Frenchs Mill, June 14, 1902; Ithaca, June 28, 1892; Karner, June 27, 1903; Keene Valley, July 16, 1894; Mount Marcy, July 31, 1913; North Chatham, June 6, 1902; Ogdensburg, July 10, 1903; Poughkeepsie, June 2, 1903; Saranac Inn, June 27, 1913; Schodack, June 22, 1902; Trenton Falls, June 18, 1898, very abundant; Wells, July 19, 1913; Westfield, June 24, 1904.

Prof. H. A. Gossard, writing from Wooster, Ohio, sent the following data:

Replying to your inquiry regarding *Miris dolabratus* will say that it has been noted as an extremely abundant and injurious insect in Ohio meadows during several different seasons. In the latter part of June, 1907, Mr. Whitmarsh took a great many specimens which are in our collection, and it must have been rather numerous. In 1912 I noticed it in such great abundance in a mixed meadow of blue grass, redtop, and timothy on the station farm that I noted it as a species worthy of special investigation, and I am glad you are undertaking the study. I noted both larvæ and adults May 11, 1912, and there are numerous specimens in our collection taken on that date. There were also numerous specimens taken June 6, 1912. I recall noting that the punctures of the insects on the grass stems were abundant but the injury to the grass was rather indeterminable. So long as rainfall is plentiful, I would not anticipate conspicuous damage from the species, but during a dry period I apprehend that it could do as much or more damage than I have yet seen the tarnished plant bug do. I certainly have never seen the tarnished plant bug in such numbers as *Miris dolabratus* during May and June, 1912. I recall that nymphs of the species were abundant and approximately mature when the earliest grasshopper nymphs were appearing in the pasture. I have not noted the species in such numbers since 1912, but possibly I have not been collecting in places that would discover it.

Prof. H. Garman, of the Kentucky Experiment Station, wrote:

Replying to your note of July 5th I have to say that my first record of the occurrence of *Miris dolabratus* in Kentucky is May 23, 1908, when it was swept from grasses in a pasture at Lexington. I have other specimens taken at Lexington, May 10, 1913. I think if the species had been common previous to the earliest date given, I should have observed it. *Oncognathus binotatus* has been common here on timothy ever since I came to Lexington. My first records of its occurrence are in 1891, about the time it was observed by Doctor Howard.

Mr. C. A. Hart, of Urbana, Ill., replied:

Our first named specimens of *Miris dolabratus* were sent us by Dr. C. M. Weed from Columbus, Ohio; I do not know the date. Our earliest specimen, the date of which

surprises me somewhat, is a well-marked female bearing the label "Hart Coll'n" and my accession #17 in my own writing. My record shows that #17 was taken at Normal, Illinois, March 18, 1883. In 1906 a specimen was taken near Urbana. We have one from Ithaca, N. Y., in 1907, July 15, and from Doctor Nason three specimens at Algonquin, near Chicago, July 9. In 1910 Davis found it at Aurora, Ill., June 15. My first real acquaintance with the species was in 1912 when I found it about ten miles east of Urbana. The next year, 1913, it was taken near Urbana May 28 and at Mahomet, Ill., west of Urbana, May 18. Another specimen is labeled May 22 from White Heath, west of Urbana. This spring I have noticed a number of specimens in grass near the University. The almost entire absence of the species from the abundant collections of the office previous to 1906 is very good proof that it was *absent or very rare previous to that year.* With us the female is invariably brachypterous and the male is macropterous. I have no notes at all concerning its food plants or other habits. We have two nymphs, both taken near here, one May 18 and the other May 28.

Both the locality and the normal date of this record are puzzling, but Mr. Hart very kindly sent the specimen to me for examination, and his well-known accuracy scarcely admits any question as to the record, though it seems impossible that a female should have been taken alive in March.

Information from New Jersey was to the effect that no records additional to those published in the Smith list of insects of New Jersey were available. Reports from other States were mainly negative.

Prof. F. L. Washburn, of Minnesota, reported specimens from Wisconsin and Minnesota, but without definite localities or dates.

Prof. C. P. Gillette, of Colorado, states that none have been obtained in their collections in that State.

Records for the Dominion of Canada, kindly furnished from the Entomological Branch of the Department of Agriculture and transmitted by Mr. Arthur Gibson, are as follows:

Ottawa, Ontario, June 25, 1908 (Gibson), June 22, 1912 (Beaulne), August 18, 1914 (Beaulieu), September 20, 1915 (Hewitt), July 14, 1907 (Gibson); Aylmer, Quebec, June 24, 1912 (Beaulieu); Chelsea, Quebec, July 3, 1909 (Groh), July 2, 1912 (Gibson), June 21, 1916 (Gibson); Montreal, Quebec, July 7, 1906 (Beaulieu); Chicoutimi, Quebec, July 24, 1915 (Beaulieu); Youghall, New Brunswick, July 5, 1905 (Gibson); Halifax, Nova Scotia, July 11–22, 1915 (Perrin). The insect is very abundant in the Ottawa district. On June 21, 1916, many were beaten from timothy (Gibson).

PROBABLY AN INTRODUCED SPECIES

With the evidence available there seems to be good reason to believe that the species was introduced from Europe at some time during the early part of the last century, probably not earlier at best than about 1800. If we may give weight to the first records by Uhler and Provancher, it is probable that the insect was introduced in New England or Quebec or some of the other maritime provinces of Canada, perhaps equally probable for Nova Scotia, New Brunswick, or Quebec. From any of these localities the dispersal might easily have reached the other

regions concerned in the course of a few decades, although without artificial assistance its progress must have been slow.

In the Harris collection, now in the Museum of the Boston Society of Natural History, I have seen specimens which had been collected in the vicinity of Boston bearing dates of 1832, 1833, 1834, 1835. In regard to these, Uhler (*1878, p. 397*) stated that—

> This species, evidently introduced from Europe, has recently become fully established in localities where it did not exist a few years ago. In Maryland, on the edges of wheat fields, and in eastern Massachusetts on grassy low grounds, it appears in swarms. About ten years ago I first met with a few individuals near Baltimore, by sweeping the grass, etc., about the edge of a wheat field; since then they have greatly multiplied, and large numbers may now be obtained there and in similar localities elsewhere. In Cambridge, Mass., the grass is sometimes crowded with them. Specimens from Connecticut, kindly obtained for me by Mr. Edward Norton, have the antennæ yellow, and are a little more slender than usual. Both the short-winged and the fully-winged varieties occur in all the localities known to me.

Evidence in favor of the species being an introduced one may be summed up briefly as follows:

(1) *Miris dolabratus* has been a common insect in Europe for an indefinite period, covering a large area and doubtless associated with the cultivated grasses to which it seems so closely restricted here.

(2) The species was not known in America until about 1830, when it was collected by Harris, as noted by Uhler (*1878, p. 397*) and also recorded by Provancher (*1886, p. 104*), although a number of careful students such as Say, Uhler, and Walsh had given no little attention to the insects of the group to which it belongs and would almost certainly have encountered it in their work in different parts of the country where it now occurs if it had been present in any abundance.

(3) It has shown a gradual westward and southward dispersal indicated by the available records, which show that it occurred in New England in 1832, Maryland in 1868, Quebec in 1872, New York in 1887, Ohio in 1888 (?), Illinois in 1906, and Kentucky in 1908.

(4) It is adapted to certain cultivated grasses which were introduced from Europe, and its close restriction to these and apparent inability to adapt itself to native grasses even of as large forms as the cultivated ones is very significant.

(5) In the plan of hibernation of eggs in stems there is evidently furnished abundant opportunity for the transportation of eggs to distant points in hay shipped for forage or packing.

DISTRIBUTION IN MAINE

The meadow plant bug has certainly been present and abundant in Maine for many years, but except for the notes by Prof. H. T. Fernald there does not appear to have been any record that assists in determining the time of its appearance or the extent of distribution. The

Experiment Station collection contains several specimens of the adult insect, the dates recorded for Orono being July 14 and 18, 1905, and July 11, 1907. Dr. Patch published a record of its abundance in 1908 and as mentioned elsewhere, I have noted it as being abundant in 1914 at Orono. Prof. C. L. Metcalf said that the species was abundant during the summer of 1916 in late instars and adult males at Fort Kent on July 5 and 6; at Presque Isle, mostly adults, on July 8; and at Houlton as adults with few nymphs of late instars on July 9. I found them abundant at Phillips and other points between Farmington and Dallas, where timothy meadows were examined, on July 18, 1916, and also very plentiful in some old meadowland in the vicinity of Saddleback Lake on July 19 and 20. None occurred on Saddleback Mountain at any point above the level of the meadowland or the growth of the timothy and other grasses commonly occupied by the species.

It is evidently safe to assign its distribution in Maine to all parts where suitable grasses occur, and it may confidently be expected to occur during the months of June, July, and August in all old meadowland where timothy forms a part of the combination, and a search in the stems will be pretty sure to disclose the eggs of the insect during other months of the year.

ECONOMIC IMPORTANCE

While, to judge from the occurrence of great numbers of *Miris dolabratus* in meadows and the evident attack on the plants, it must be inferred that the insect causes serious injury to the crops, there appears to be little to establish the amount of loss or to separate it from that due to other species. In fact, but few of the Capsidae have been given much attention from the economic standpoint. The familiar and cosmopolitan tarnished plant bug, *Lygus pratensis*, has been known for years as a pest to many plants. In 1892 Howard (*1892*) called attention to *Oncognathus binotatus* as "a new enemy to timothy grass." Dr. M. V. Slingerland, of Cornell University, has treated the common 4-lined plant bug (*Poecilocapsus lineatus*) as a pest of currants; Prof. E. A. Popenoe, Kansas Agricultural College, has called the little *Halticus citri* (Ashm.), a garden pest of beans; and the common *Adelphocoris rapidus* has been known for many years to affect the clover crop.

Some idea of the effects produced by the meadow plant bug may be obtained by noting the enormous numbers to be found hanging to the plants and especially to the heads during the time the timothy is in bloom. Often a number cling to a single head, from three to five being not unusual. The fact that they suck the bloom doubtless means a heavy loss in seed or in weight and nutritive value of hay, although there is little external evidence of injury.

Evidence of injury based on the amount of hay per acre where the meadow plant bug is plentiful as compared with fields where it is absent,

suffers from the fact that so many different insects are present and it is almost impossible to determine the proportion of damage to be charged to each. To judge merely by the numbers present and also by the size and feeding capacity of *Miris dolabratus*, it may easily be counted among the most destructive to the crop, though it does not kill the plant by attacks at or near the root.

FOOD PLANTS

Timothy has been most commonly mentioned as the food plant of *Miris dolabratus*, and this is quite evidently the grass with which it is most commonly associated, as even where it may be found on other grasses it is usually where timothy forms a large part of the combination of species growing together.

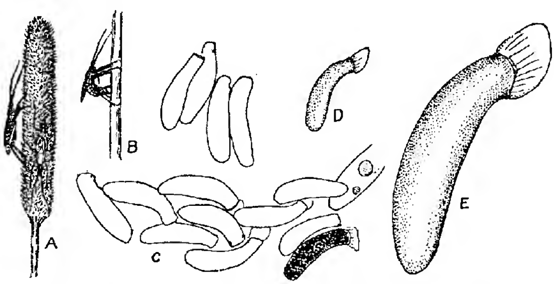

FIG. 1.—*Miris dolabratus:* A, adult on timothy head in resting or feeding position; B, female ovipositing; C, eggs from oviduct, nearly or quite mature; D, mature egg ready for deposition; E, mature egg greatly enlarged showing membranous operculum.

I have found it commonly on timothy heads, very evidently feeding, and individuals have been carried along for several instars with no other food; hence, it is clearly a normal food supply (fig. 1). I have also found it commonly on orchard grass (*Dactylis glomerata*), meadow fescue (*Festuca elatior*), and witch grass (*Panicum capillare*) and the nymphs seem to thrive on these plants about as well as on the timothy. The meadow plant bug has not been observed commonly on bluegrass (*Poa pratensis*) or other small grasses or grasses with small seed heads, except as these are mixed with the coarser forms. While it occurs where clover is mixed with timothy and lays eggs in clover stems, it has not been observed ever to feed on stems, leaves, or heads of clover. I believe it is quite strictly a grass-feeding species, primarily adpated to timothy.

Before the grasses head out, *Miris dolaratus* is found on the stems and leaves, but the larger part of the growth of the insect occurs after timothy begins to head. The heads seem to be the favorite point of attack. The insects cluster on the heads sometimes in numbers to a single head and thrust their beaks down into the flowerets, evidently drawing their

food from the tender parts of the blossom or from the forming seed. I have seen a female thrust her beak down in the flower of witch grass, piercing the glumes, or insert her beak between and down into the anthers, penetrating them and causing them to burst, and probably sucking juices from the ovules.

Slosson's (*1894*) record for Mount Washington above 5,500 feet is for adults, and Mr. C. W. Johnson informed me that many insects capable of flight are carried up by air currents and found at altitudes much above that of their food plants.

SYNONYMY

The abundance, wide distribution, and variability of the meadow plant bug may be inferred from the many names which have been applied to it by different writers. No less than 13 specific names have been given to it, and with the various generic combinations used this number is still increased.

For the detailed statement of the synonymy I am indebted mainly to the exhaustive catalogue of Oshanin (*1909, p. 778*), whose records are almost exclusively European. A more complete bibliography is given by Van Duzee (*1917*). Its synonymy according to Oshanin is as follows:

MIRIS FAB. REUT.

Miris Fabr. S. R. p. 253 (prt.); Reut. Rev. Syn. p. 243; Hüeb. Syn. Blindw. 1, p. 33 et 63; *Leptopterna* Fieb. Cr. Phyt. p. 302; Eur. Hem. p. 63 et 244; Reut. Gen. Cim. p. 9; Rev. cr. Caps. 2, p. 13; *Lopomorphus* Dgl. Sc. B. H., p. 293. *Lopus*. Herrick Schaeffer. Wanz. Ins. III., p. 35.

dolabratus Lin.

Cimex dolabratus L. Syst. Nat. ed. 10, p. 449 [1758]; ? *Cimex frumentaruis* Poda Ins. Mus. Graec., p. 60 [1761]; *Cimex riparius* Scop. Ent. carn., p. 135 [1763]; *Cimex laevigatus* Deg. Mém. 3, p. 292 [1773]; *Cimex lateralis* Fabr. Gen. Ins., p. 300 [1776]; ? *Cimex deses* Müll. Zool. Dan., p. 108 [1776]; *Cimex antenni-rectus* Goeze Ent. Beitr. 2, p. 267 [1778]; *Cimex V-flavum* Goeze ibidem, p. 279 [1778]; *Cimex porrectus* Geoffr. in Fourcr. Ent. Par., p. 206 [1785]; *Cimex recticornis* Gmel. Syst. Nat. ed. 13, p. 2185 [1788]; *Miris abbreviatus* Wolff Wanz. f. 110 (♀) [1802]; *Miris lateralis* Wolff Wanz. f. 109 (♂) [1802]; ? *Miris picticeps* Curt. Brit. Ent. 15, t. 701 [1838]; *Miris dolabratus* Hhn. W. I. 2, p. 75. f. 160 [1834]; Flor. R. L. 1, p. 437; Reut. Rev. syn. no. 209; Hüeb. Syn. Blindw. 1, p. 66; Atk. Cat., p. 34; *Lopus id.* H. S. W. I. 3, p. 45, f. 261 et 262 [1835]; Mey. Caps., p. 38; Shlb. Mon. Geoc. p. 88; Kbm. Caps., p. 196; *Leptopterna id.* Fieb. Eur. Hem. p. 245 (prt.) [1861]; Reut. Rev. cr. Caps. 2, p. 15; Saund. Synops. 2, p. 262; Prov. Faun. Ent. Can. Hém., p. 104 [1886]; Saund. Hem. Het. Br., p. 227, t. 20, f. 10; *Lopomorphus id.* Dgl. Sc. B. H., p. 297 [1865]; Uhler, Bost. Soc. N. H. XIX, p. 397. 1878; *Miris Belangeri* Prov. Natur. Canad. 4, p. 78 [1872].

DIMORPHISM

The species occurs in two distinct forms of females, a long-winged and a short-winged form; but only one form of male, the long-winged, has been observed. The short-winged form of female is by far the most abundant; and as this form is entirely unable to fly, and therefore is very

definitely restricted in its migration, it is of special interest to note that it must be the form which produces pratically all of the eggs.

In the short-winged form the elytra reach only to the fifth abdominal segment leaving a large part of the abdomen, especially when engorged with eggs, conspicuously exposed. While there is some variation and occasional intermediate forms, there is a great preponderance of individuals with elytra uniformly about 5 mm. long and with the wings still shorter. None have been seen with the wings entirely aborted.

The relative proportion of eggs produced by the two forms will be discussed under the head of egg production, but it may be said here that from collections made during the summer of 1916 it appears that only about 10 per cent of the females are long-winged and that these produce a much smaller number of eggs each than the short-winged forms.

The biologic significance of the dimorphism can hardly be entered on here. It is, however, distinctly similar to what occurs in many other of the Hemiptera and doubtless depends on some fundamental factors in food supply, seasoned migration, or other adaptation.

It offers many interesting biological problems for investigation and naturally presents some most essential elements in the consideration of general control.

LIFE HISTORY

The eggs hatch in May or early June, the time being determined in part by latitude and season. The exact date of hatching at Orono was not observed, as nymphs were already abundant at the time of my arrival on June 12, and as the season of 1916 was exceptionally late, it is probable that the average date of hatching would be the last week in May. Young nymphs continued to appear until about June 25, but none hatched after July 1. The first adults appeared on June 16 and were abundant by June 26.

Evidently the adults feed for some time before mating, as the first matings observed were on July 8 and 10. The eggs, however, develop rapidly when the insects reach the adult stage, since fully developed eggs in large numbers, 50 to 60 to the individual, were dissected from the females, the first one dissected, on June 30, containing 30 eggs fully formed, as well as others in an immature state. Another, dissected on July 8, contained 69 developed and a few immature eggs.

These dissected eggs were of special interest, as they might furnish the clue to a later determination of place and method of oviposition, as the peculiar strongly curved neck and large membranous expansion over the head naturally suggested some rather unusual mode of placement.

The nymphs cling closely to the plants and while they pass readily up and down the stems and doubtless shift from one plant to another there is no extended migration, probably no movement providing for any dispersal. When molting, they cling to the plant and, as with other

insects generally, the skin splits along the middle line of the back and the body and legs are gradually withdrawn and the increase in size and resumption of color takes place in a short time.

Five distinct stages of the nymphs are recognized and this seems to be the general rule for the Hemiptera, being the number noted in a large number of the species which have been reared through the nymphal stages. These will be described in detail in a later paragraph (fig. 2).

No single individual has been carried from the first instar through to the adult stage, but numbers have been carried from two to four of the instars in confinement and under observation so that it is possible to give a connected series of stages from the smallest found to the adult

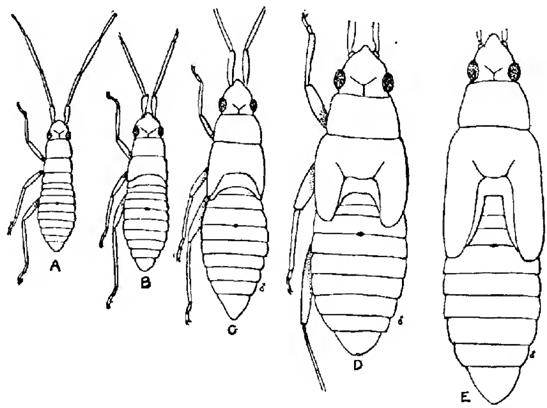

FIG. 2.—*Miris dolabratus:* Nymphs showing relative size of body and development of wing pads. A, First instar; B, second instar; C, third instar; D, fourth instar; E, fifth instar. Note also position of dorsal gland orifice between segments 3 and 4. (Original.)

form. The time occupied in the different stages has run from 5 to 8 days, averaging 6 to 7 days, and the total period of development from hatching to adult stage must be about 30 to 35 days.

The principal changes are in the increase in size and in the growth of the wing pads, which are entirely wanting in the first, appear as faint enlargements of the mesothorax in the second instar, are fairly distinct on both mesothorax and metathorax in the third, extend to the second abdominal segment in the fourth and on to the middle of the fourth segment in the fifth for the female and to the base of the fifth segment for the male. There is considerable irregularity in development, as instars 1 to 4, and probably 5, with adults were taken on June 23.

In the fourth and fifth instars the sexes are easily distinguished, males being slightly narrower, the abdomen with more parallel sides, and the

genitalia being seen in outline through the semitransparent walls (fig. 3, A, B).

With the final molt the wings expand rapidly, and the distinction between males and females and the dimorphic forms of females become clearly marked.

The adults remain quite constantly on the grass heads and evidently feed for a number of days before mating or egg laying begins.

The insects cling to the stems or heads of grass quite firmly, the tarsi, which are quite long, being evidently well fitted for securing a firm hold to the parts of the grass. They seem to obtain the best hold on the smaller stems, 1½ to 2 mm. in diameter. On the timothy heads the tarsi catch in the florets, which seems to give them a very firm foothold, as they are not easily dislodged. They could doubtless cling to the heads easily while the hay is being handled.

On the grass heads they are more commonly found with their heads directed upward, and they show some tendency to mount to the highest

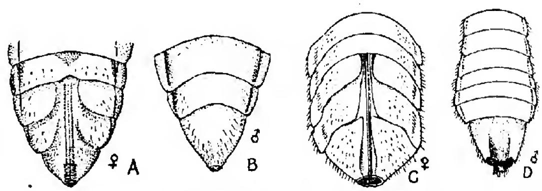

FIG. 3.—*Miris dolabratus*, genital segments: A, Female; B, male of fifth instar nymph; C, female; D, male of adult. (Original.)

point; but they often stand head downward and pass up and down the heads and stems freely. The position with the head upward appears to give them an excellent position for probing into the florets with their beaks. It was noted that in egg laying the females selected small stems of grass, 1 to 1½ mm. in diameter, while in clover stems as much as 2½ mm. diameter were used. Probably the rough or softer stem of clover serves as a good foothold, though the tarsi are too small to clasp around it.

The insects are not very conspicuous on the grass, even where plentiful, and may be somewhat protected by their form and coloration, especially from a little distance. At close range they are disposed to dodge behind the grass when disturbed, and they can move with considerable celerity.

The proportion of the sexes and the two forms of female is of interest and possibly of economic significance, since it bears very directly on the ability of the species to spread from the fields in which they hatch.

In the ordinary field captures the short-winged females were in the majority, and in captures with special effort to obtain all forms they were usually somewhat more numerous than males, a fact that may be

due to the greater activity of the males which enables them to avoid the net. The disparity is not so great, and a fairly equal proportion of males and females may be assumed. For the macropterous and brachypterous forms of the female, however, there is very evidently a decided disproportion, amounting in the captures at Orono to about 1 to 9—that is, 90 per cent of the short-winged to 10 per cent of the long-winged. Of 125 collected on July 11, 51 (40.8 per cent) were males and 74 (59.2 per cent) females. Of the females, 72 were short-winged and 2 long-winged.

This disproportion seems to be still further magnified by the greater number of eggs developed in the short-winged forms.

There is some reason to think that the long-winged females develop slightly earlier than the short-winged ones, and possibly there is some scattering of these and an earlier egg deposition; therefore observations to correct or verify this point are desirable.

As usually found in the field, the brachypterous forms were much more evident and undoubtedly constitute the main source of egg production. They usually show much greater distension of the abdomen, and are full of well-developed eggs at time of mating. They are so heavy they would probably fly with difficulty even if the wings were not aborted. As it is, they can not fly at all, make no attempt to use the wings, but drop or flutter helplessly to the ground when thrown into the air; hence they must necessarily lay their eggs in the immediate vicinity of the place where they have hatched and grown. No mating of long-winged females was observed, but this is not strange and does not warrant the assumption that they are unfertilized.

EGG PRODUCTION

The dissection of a number of individuals when fairly mature as well as the number of eggs deposited by individuals under observation indicates an egg production of from 60 to 70 for each brachypterous female. One dissected macropterous female contained 60 eggs, but all others had from none to 15. Of those dissected on July 11, 27 contained a total of 58, 21 had none (it is barely possible they had completed oviposition), 4 had 10 or more, the highest being 15 each.

If these figures can be taken as at all representative of average conditions, it means that on the basis of an average of 70 eggs per individual for the brachypterous and of 2 for the macropterous form, and assuming that 10 per cent were macropterous, the macropterous would produce but one three hundredths of the eggs, which would be an almost negligible number as affecting dispersal and would raise some interesting biological questions concerning the survival of the brachypterous form. However, as already hinted, these figures must be considered as representing a very limited period and were obtained before we had sufficient acquaintance with the species to secure proper checks.

MATING

The preliminary steps in mating have not been observed, but in a number of cases males and females in copulation have been kept under observation for several hours. The usual position is for the male to be at the right side of the female with the legs of the left side clasping her body, the forelegs crossing the forward part of the body and the others disposed across the thorax and base of the elytra. The right legs remain free and may be used slightly in clinging to adjacent objects, but the female alone may cling to the grass head or stem. The tarsi of the left legs of the male are held against the margin of the female's body and apparently kept in one position for the entire period of mating. The abdomen of the male is bent under the female with its dorsal face uppermost and the genital organs inserted at the base of the ovipositor.

In one instance a pair taken on July 10 about 7.40 a. m. and carried on the grass stem for about a mile remained in copulation until 9.40 in spite of considerable handling. After separation they showed no inclination to subsequent mating. The male died on the third day following, and the female began ovipositing in about 24 hours after mating, certainly then and possibly earlier. Other observations confirm the view that mating lasts for several hours, and no evidence has been found that either males or females mate more than once.

Considering the tenacity of their hold and the tendency to cling closely to the grass while mating, it is possible that methods for capturing them might be more effective at this period. It is quite possible that they might be carried with hay at this time more easily than otherwise, and if scattered in favorable situations serve to provide for wider dispersal

EGG DEPOSITION

The process of egg deposition is interesting and has been watched repeatedly with females confined in glass tubes, but it has not been observed directly in the field. The females in the field evidently succeed in keeping out of sight or perform their egg laying with such rapidity that there is little chance of finding them at work. It was only after watching them in confinement and learning how the eggs were laid that we succeeded in discovering the eggs in stems in the field where the insects had been abundant.

When about to oviposit, the female seeks a suitable place on the stem, more frequently with the head upward, but often in the reverse position, and explores the stem carefully with the beak. She appears to make a slight puncture with the beak then draws the abdomen at a sharp curve up under the thorax and places the tip of the ovipositor on the spot where the beak has rested. The ovipositor is withdrawn from its sheath and stands at nearly right angles to the genital segment and the insect with all legs clasping the stem and evidently strained very tensely begins

a swaying movement forward and backward working the tip of the ovipositor slowly into the stem. If unable to start it promptly, she may shift her position and reinsert the beak, then begin again with the ovipositor. When the point of the ovipositor has been thrust in, evidently at the point where it has penetrated the outer wall of the stem, the body is pushed forward and the ovipositor pushed strongly downward and backward till it is embedded its full length. A few contractions of the abdominal segments serve to slip the egg along the ovipositor, a scarcely visible operation from the outside, and the ovipositor is quickly withdrawn. The slit in the stem closes up so as to be entirely invisible. The egg is held by an operculum. The insect takes a short interval of rest or may renew the process almost immediately, eggs being laid at the rate of one every minute or minute and half to two minutes. On the withdrawal of the ovipositor, she feels with her beak the point where the egg was inserted, moves forward a trifle, and again feels the surface with the beak, apparently relaxes a little and then, rubbing the fore tarsi together and holding them free, vibrates the joints as if to limber up after the severe exertion of forcing the ovipositor into the stem, and proceeds to lay another egg. About 20 eggs have been laid in the course of half an hour, and this is about the highest number usually found in a series in stems collected in the field.

That eggs are laid on different days was shown by one female which laid about 20 one afternoon between 4 and 5 o'clock, 11 on the following day at about the same hour, and 6 the third day between 4.40 and 4.50 p. m. She may have laid others when not under observation, as 52 eggs were later counted in the stems in which she had oviposited, and these were pretty surely all laid by this one female. As she was dead on the morning of the sixth day after the first oviposition had been seen, she may have laid eggs possibly on four or five days, but certainly most of them laid after she was under observation must have been deposited during three days. It is rather striking that the eggs should be laid on successive days at so near the same time of day. In other instances oviposition was observed in the morning, but the time of day may be fairly uniform for each female. This long interval affords the insect an opportunity to rest, probably to feed, and may also be associated with the maturing of the eggs or their adjustment in the oviducts.

The insects disappear rapidly after the egg-laying period, so quickly in fact that we were much puzzled as to their whereabouts. An examination within a day or two, especially after the grass had been cut, would show scarcely an insect where they had been numerous; therefore a special effort was made to follow them after the mowing. The males, of course, could fly readily, but they show a tendency to cling pretty closely to the grass and might be carried from the fields with the hay. The females cling still more closely to the grass, the short-winged ones making no attempt to fly, and the long-winged ones having apparently

very little disposition to travel in that way. After the cutting of the grass, the females would be found running about over it, no males nor long-winged females being observed. Mr. Fletcher reports close watching of one female for an hour and a quarter. She ran about over the cut grass, but did not attempt to get beneath it nor hide in any way, except when a sudden movement by the observer took place. Then the insect would dodge behind or under a grass stalk, where it would remain for a few minutes. It was not observed to feed during the time it was watched. In another field, an old meadow, a careful examination was made, Mr. Fletcher reporting that—

> A course was taken from the outside swath to the inside one so that grass cut for about two hours and freshly cut grass was examined. Females were found easily upon the top of the grass; but no males. Two long-winged females observed acted to all appearances like the short-winged individuals, making no attempt to fly. The females made no attempt to seek shade, though the sun was high.

However, the insects ordinarily cling to grass heads in full exposure to the strongest heat of the sun.

After the hay has been raked together, it is practically impossible to find insects in the stubble, however, they may be found for a time on the haycocks, but not down in the hay. Some of them may very probably be carried in with the hay to the haymows, but are not to be found by an ordinary search.

The most plausible explanation for this very rapid disappearance is that the insects die shortly after egg deposition, and that as most of the females had oviposited before the grass was mowed, they died off rapidly afterwards, and their shriveled bodies became difficult to find or were disposed of by ants. Their bodies are very fragile, and easily broken to pieces when dry; hence, a few days of dryness, or possibly a heavy rain, may destroy nearly all traces of the dead bodies.

Those that have not deposited eggs can evidently carry this on readily in the stubble, as it has been determined that the great majority of the eggs are placed near the ground.

It is entirely probable that females may be carried in with the hay and deposit eggs in the stems after the hay has been stored. Where the hay has been cut early or before the time of egg deposition, this is likely to occur; but from the apparent efforts of the insects to keep above the mass of hay it seems probable that the majority of those covered deeply would fail to oviposit. It is doubtful whether they could oviposit in dry stems, and even if they did, the fate of the eggs would seem to offer little opportunity for the serious dispersal of the insects, except where shipped to a distant locality.

It is conceivable that, considering the evident toughness of the egg-shells and their ability to resist adverse conditions, the eggs might pass through the alimentary canals of horses or cattle undigested and uninjured, and later possibly be carried back to fields in the form of manure;

but such a transfer, even if possible, must have little, if any, practical importance, considering the normal condition of the great mass of eggs being placed near the ground and protected for their period of development in the stubble.

PLACE OF DEPOSITION

In order to determine as certainly as possible where the majority of the eggs are deposited, counts were made of a number of stems of the grasses on which the adults commonly occur and from a field where the insects had been present in large numbers during the present summer. One hundred stems each of timothy, orchard grass, meadow fescue, and witch grass were selected from a point where the insects must have been abundant and where eggs should certainly be plentifully found. Out of this number two timothy stems were found with eggs, one of meadow fescue, and one of orchard grass, but the witch grass had none. An additional 25 stalks of timothy were split, and many stalks were examined and slit part or all of their length without finding any eggs in the upper part of the stem. In no case were eggs found beneath leaf sheaths or in stems where it would have been necessary to puncture through the leaf sheaths to reach the stem.

It appears very clear, therefore, that the great majority of the eggs are placed in the stems of plants near the ground and below the level of cutting, so that but very few can be carried out of the field with the hay in cutting or harvesting. This makes very clear the presence of immense numbers of insects in meadows which are regularly mowed and from which the hay is removed, and emphasizes the effect of the short-winged condition and inability to fly of the greater proportion of the females. However, a sufficient number of eggs may be placed in the stems above the height of the stubble or be raked up in stems from the ground and carried in with the hay to provide for limited dispersal and account for the transportation of the species from one region to another (Pl. 12).

DESCRIPTION OF MIRIS DOLABRATUS

No very satisfactory description of *Miris dolabratus* is available in the accessible textbooks or manuals.

The adults are about two-fifths of an inch long (9 mm.), rather slender, with long black antennæ which are thickest at the base, the head is rounded, set fairly close to the thorax, which widens behind; and the wings lie nearly flat on the back, are narrow, and have nearly parallel sides, extend to or slightly beyond the tip of the abdomen in the males and long-winged females and to the fifth abdominal segment in the short-winged females. The color is yellow or yellowish gray with dark markings, which form two rather indefinite stripes on the pronotum and elytra. The antennæ and legs are black, with yellowish bases or yellow with black hairs and spots.

The nymphs are yellow marked with black, the general color being quite dark in the early instars and becoming lighter with the successive molts.

DESCRIPTIONS OF EARLY STAGES

THE EGG

The egg of *Miris dolabratus* is of quite unusual shape and shows a quite remarkable adaptation to its anchorage in the stems of plants. It is of elongate-oval and strongly curved especially near the head or attached end, which expands slightly from a constriction in the curved part and is surmounted by a large flat chorionic membranous expansion or operculum, the width and length of which is nearly one-fourth that of the egg itself (fig. 1). The egg is shining, polished with a dense chorion, and when placed in the stem with the operculum held firmly in the wall stands out into the hollow of the stem. In clover stems they may be embedded partly in the pithy layer lining the stem. The eggs are fully developed in the ovaries soon after the adults mature and show fully the enlarged opercula. The position of micropile and the route of entry for the spermatozoa is undetermined, though it would seem almost necessarily through the operculum. Egg length, 1.35 mm., diameter 0.25 mm. (fig. 1).

FIRST INSTAR

The smallest individuals found, and evidently first-instar individuals partly grown, were 2.25 to 2.50 mm. long. The color is quite dusky or blackish, the antennæ and legs being entirely black, except a slight pale portion of membrane at joints. The head above is blackish with yellow area next the eye; beneath it is yellow. The thorax is yellow, with a broad black stripe on either side; or it may be black with the median and the marginal line yellow. The abdomen is brown, the lateral stripe being yellow, and an oblique patch on each segment is yellow. Beneath the abdomen is brown, with the hinder border of segments yellow.

The antennæ have a total length of 2 mm.; the second and third segments are equal in length, slightly longer than the fourth and twice as long as the first, the fourth being about as thick as the third. The beak extends to the hind coxæ and is thick.

SECOND INSTAR

The insect in the second instar resembles the first in color, being dusky though of a trifle lighter color and showing more of the greenish gray of the later stages. This color becomes more pronounced as the insect grows during the instar, especially for the margins of the thorax and abdomen. The wing pads are very slightly indicated as blunt lobes on the hinder borders of mesothorax and metathorax. The antennæ are

distinctly longer, the second segment being longest, with the third a little longer than the fourth. The beak reaches the base of the hind coxæ. The length is 3 to 3.5 mm. and length of antennæ 3 mm.

THIRD INSTAR

The third-instar individual is distinctly lighter colored, and the pattern of marking is more like that of the later stages. The wing pads are distinctly indicated, those of the metathorax extending well onto the base of the second abdominal segment and those of the mesothorax extending well back on the metathorax pads to a line with the base of the second abdominal segment. The antennal segment, too, is nearly one and a half times the length of the third, the third twice the length of the fourth and three times the length of the first. The beak reaches between the mid and hind coxæ. Length, 5 mm. Total length of antennæ, 4.5 to 5 mm.

FOURTH INSTAR

Lighter in color, greenish border of thorax and abdomen broader, first and second segments of the antennæ, except at apex greenish; legs except tarsi greenish, hairs black; antennal second segment about one and one-half times as long as the third, the third three times as long as the fourth and first, fourth and first about equal. The wing pads are considerably enlarged, the mesothoracic pads extending over and beyond the metathoracic pads and to the middle of the third abdominal segment. The beak reaches to the hind border of the middle coxæ. Length of body, 6 mm.; of antennæ, 6 to 6.5 mm.

FIFTH INSTAR

The individual of the fifth instar is light gray-green or yellowish green with black or fuscous patches and stripes forming two nearly continuous stripes over the wing pads and abdominal segments, paralleling the margin, and a median double row of spots on abdomen; antennæ greenish, tips of first and second and all of third and fourth segments blackish, legs greenish, tarsi black. Antennal segment two three times as long as the first, two and one-fourth times as long as the fourth, and about one and two-thirds times as long as the third. Length of body, 6.75 to 7.25 mm.; of antennæ, male, 6.25 mm. The beak extends to the base of the middle coxae.

The characters that seem of special service in recognizing the various instars are the comparative lengths of the antennal segments and the development of the wing pads. While these are subject to slight variation in different individuals, they seem fairly constant, and the descriptions and figures have been made from what seemed to be representative specimens. The orifice of the dorsal gland between third and fourth segment, while not furnishing distinctive characters of the instars, is a good landmark for locating the abdominal segments.

The adult antennæ are much longer proportionately, and this elongation is due mainly to elongation of first and second segments, the first is one-third of the second, the second nearly four times the fourth and nearly twice as long as the third (fig. 4).

NATURAL ENEMIES

Among the natural enemies or checks of the species may be counted the gray-damsel bug, *Reduviolus ferus* (fig. 5), which is a very common and widely distributed species throughout the northern part, at least, of North America, and in fact the holarctic region. Webster and Mally (*1897, p. 41*) states that *Coriscus ferus* was observed to attack *Leptoterma dolobrata* L. which was feeding quite abundantly on timothy heads about East Cleveland, Ohio, on June 28, 1896.

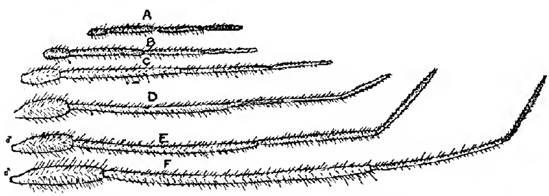

Fig. 4.—*Miris dolabratus;* A-F, Antennæ of nymphs: F, antenna of adult male drawn to same scale and showing relative lengths of segments. (Original.)

This species has been found in Maine associated with *Miris dolabratus* as well as with the leafhoppers, and it is probable that it may feed on either with equal avidity. While it has not been seen feeding on the mature *M. dolabratus*, it is pretty certain that it will feed upon the larvæ, as its fondness for leafhoppers in both the nymph and adult stages has been proved by repeated observation. Direct observation of the insects attacked is difficult, as it is almost impossible to follow them in their movements in the field. Even when offered a variety of food in confinement it is difficult to determine their selection in the species offered, as they very seldom make their attacks on the insects while under observation, and it is left to infer from the numbers killed how ready they are to prey upon different kinds. We have had them live and thrive in confinement and progress to the adult stage on a diet of *M. dolabratus* and also on a food supply of leafhoppers, and it is probable that they will eat a variety of small insects, the particular kinds being determined rather by the available supply than by any definite choice on the part of the bug.

They are able to survive considerable periods without food, and their development is doubtless affected either by abundance or scarcity.

The appearance of the different instars of the early stages is not at all uniform, as individuals of several different stages, as well as adults,

may be found at the same time in the early part of summer. Thus, a first-instar individual was taken on June 27 and a second-instar on July 5, a third on July 6; but fifth-instar individuals occurred on June 26; one changed to adult on June 30, and the other fifth-instar individuals were taken on July 6 and 13. Mr. Fletcher collected 10 nymphs, last instar, and 33 adults, 9 males and 24 females. During the latter part of the summer only adults are found, so it appears that the early stages must be passed during spring and early summer. During the earlier part of the summer fifth-instar individuals were taken much more commonly than the earlier stages, and it would seem that they remain in this stage longer than the earlier instars, or else they are in position for a more ready collection. Confined and furnished occasional leafhoppers as food, they have been carried for a period of seven days in this stage.

Adult females contain well-developed eggs in late summer, and while there is no probability of a second generation, at least in the latitude of Orono, it appears probable that eggs are laid in autumn to hatch the following spring.

The smallest individuals found and evidently of the first instar are of a very delicate, almost transparent whitish color, with a length of 2.5 mm. and a width of thorax of about 0.6 mm. The hind tibiæ are 1.5 mm. The antennæ have four joints: First thickest, second slightly longer than the first, the third the longest, a trifle longer than the second, the fourth about equal to the third. The eyes are red. A conspicuous red line is seen on the thorax and abdomen, with a dusky stripe at the side of the thorax (fig. 5, A).

The second-instar individuals have a length of 4 mm., with antennæ of 3 mm. A conspicuous dark-red median line runs from the head to the tip of abdomen. A broad yellow stripe occupies the middle of the body, bordered by a dusky, irregular stripe each side. Legs light yellow spotted with black, tarsi black at tip. The wing pads very slightly indicated at the outer angles of mesothorax and metathorax (fig. 5, B).

The third instar has a length of 4.5 mm. and is a little thicker than the second. The red line along the median dorsum is conspicuous, but somewhat broken and at places very slender. The antennæ are dark except tip of first and basal four-fifths of the second segments (fig. 5, C).

No specimens considered as representing the fourth instar have been seen.

The final observed nymphal instar and apparently the fifth has a length of 6 mm., a width at base of abdomen of about 2 mm., and is elongate-fusiform in shape. The head is slender and the eyes globose. Antennæ slender, the first joint thickest, minutely hairy, second and third about equal in length and slightly longer than first or fourth. Forelegs with the femora enlarged, middle femora nearly as large as the fore, the hind femora slender. The beak is 3-jointed and reaches

to base of the first coxæ. The color above is olive, with a central spot on segments 3 and 4 and the margins of the abdomen yellow or salmon-colored, tinged with orange. A dark-red or red-brown median line runs from the head to end of abdomen interrupted on base of meso-thorax and

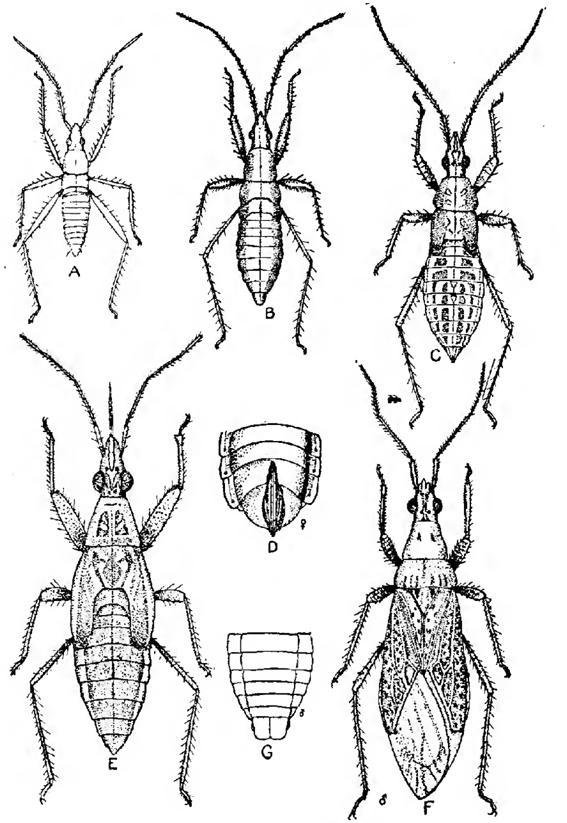

Fig. 5.—*Reduviolus ferus* L.; A, first instar; B, second instar; C, third instar; D, genital segments of female; E, fifth instar; F, adult male; G, genitalia of male.

at abdominal articulations. There are dark spots of various designs on the head, prothorax, mesothorax, and wing pads. The wing pads are dusky at tips; legs with black dots; tarsal tips black. Yellow spots occur on head, thorax, and a marginal series on abdomen in outer part of the olive

area. Beneath olive and yellow, the former mainly in form of a broad median stripe including some yellow spots. The pectus is mainly yellow, the abdomen yellow (fig. 5, E).

Reference has been made to the record of Leonard (*1916*) of rearing *Phorantha occidentis* from *Miris dolabratus* in New York. A larva, apparently a tachinid, was obtained issuing from a nymph of *M. dolabratus* at Orono, June 17, 1916, but it failed to mature. On July 18, 1916, a nymph which issued from *M. dolabratus* buried itself promptly in the earth and from this an adult emerged on July 29. This larva issued from the anterior part of the abdomen under the wings, while the host was still alive. The species has not been determined, but is evidently closely related to the species bred by Leonard.

Spiders are undoubtedly quite efficient enemies of the plant bugs, but they dispose of their prey so completely that little is left as evidence of their work. Mr. Fletcher found an individual of *Miris dolabratus* encircled by a spider's web in which it had evidently been captured and enmeshed. In some unpublished records made by Mr. Sherman Bilsing in Ohio a number of different species of spiders are credited with feeding on this species, along with many other capsids.

The extent to which birds, toads, and other animals may serve to reduce their numbers is open to study.

The Entomophthorae may be considered as potential checks, but so far no extensive destruction of *Miris dolabratus* has been noted by them. A dead specimen infested with fungus was collected by Mr. Newman on June 28, 1916. This was sent to the Bureau of Entomology and referred by Dr. Howard to Mr. A. T. Speare. His report is as follows:

> The single specimen of *Miris dolabratus* infected by a fungus closely related to the above (*Entomophthora aphidis*), but, owing to insufficient material, I would not venture to assign it anything but the generic name Entomophthora. Both contributions are very interesting, the latter especially forming, I believe, a new host for this group of parasites.

CONTROL MEASURES

While the main efforts in the studies during the summer of 1916 have been directed toward solving a number of puzzling questions as to the development and habits of the species, and while some definite experiments are needed with certain measures that may be suggested on the strength of the facts learned, there is certainly a very definite basis established for certain kinds of treatment that should do much to reduce the numbers of the insect.

The most fundamental point determined with reference to the insect in connection with control is doubtless the fact that the eggs are deposited in the stems of plants in the fields where the insect has been present. This, in connection with the fact that the great majority, probably 90 per cent of the females, are wingless, means that we know that practically all the eggs deposited in midsummer are in the old timothy meadows

and that any measure which will destroy them in this location will have immediate effect in preventing further loss from this source.

It is very evident that plowing under and planting of the field to a different crop will absolutely prevent further injury from the stock of insects established in any old meadow, and therefore rotation, where this is practicable, may be counted a certain remedy for the field concerned. However, for the protection of adjacent fields or in order to exterminate the insect as completely as possible the borders of the fields and the fence rows usually supporting a considerable growth of grass should be remembered and, for the disposal of this insect, should be plowed as closely to the border as possible or burned over when the grass is dry, so as to destroy the eggs as completely as possible.

Where rotation is impracticable or undesirable, it will be more difficult to obtain complete eradication, and careful tests of treatment, based on the habits of the insect, are necessary to determine the most successful methods.

It is clear that burning over of meadows if sufficiently dry in autumn or early spring so as to destroy the eggs would be very effective, but there are, of course, many objections to this treatment so that it can not be urged as sufficient. In some seasons probably there would be no time when the grass would burn sufficiently close to the ground to destroy any large part of the eggs and there is the danger, if burned too deeply, that the stand of grass will be injured. This method, especially for the conditions prevailing in Maine, does not seem to promise much. Where burning is practiced, it should assist. It would be worth while to compare results in field so treated.

Early or late cutting of the crop may have some effect on the number of eggs laid in a field, an early cutting, before the insects are mature, for example, depriving them of their usual form of food, the heads of grass, may reduce egg deposition, but whether to such an extent as to warrant any special change in the usual practice as to time of cutting can only be determined by further study.

The application of any form of insecticide or of special kinds of fertilizers does not seem to offer any very practical relief, and the use of hopper-dozers or mechanical devices for their capture have not been tested; nor do they have much promise.

Finally there is the important consideration of the spread of the insect into adjacent fields or farms or to more distant points, and for this the facts obtained furnish a very sure foundation for effective control. Since practically the only opportunity for such wider distribution is by carriage of hay, the disposal of any such material introduced where the insect is not present in some way so as to avoid scattering the eggs where they can hatch where suitable food plants will be available for their subsistence will serve to exclude them.

SUMMARY

(1) *Miris dolabratus* has been a conspicuous insect in timothy meadows in portions of the eastern United States during the past 40 years and now has a distribution as far west as Illinois and Minnesota and south in the Mississippi Valley into Kentucky.

(2) It is believed to be an introduced species, coming from Europe with timothy hay or other large-stemmed grass shipped for forage or packing some time between 1800 and 1825.

(3) It feeds upon cultivated grasses, especially timothy, orchard grass, and meadow fescue, and when abundant must seriously affect the value of the crop.

(4) It is a dimorphic species, there being two forms of females, a long-winged and a short-winged form, the latter being far more plentiful, about 90 per cent.

(5) The species hibernates in the egg form; hatching occurs about May 25 to June 10 in Maine; and the nymphs pass through five instars of about six or seven days each, adults occurring from early July, mating and laying eggs from July 10 to August 1 for the short-winged forms necessarily in the fields where the females have developed.

(6) The eggs are laid in stems of grass or clover in fields where females have grown, being thrust through the wall of the stem and held by an expanded cap which is firmly held by the walls of the stem, the egg being protected in the hollow of the stem and in this position remain for at least eight or nine months before hatching.

(7) Measures for control so far evident and based on habits determined will consist especially of rotation, with probably some advantage from burning, early cutting, pasturing heavily in fall, and possibly by mechanical devices for capturing the nymphs or adults.

(8) The spread of the insect should be prevented by care in the disposition of timothy hay moved to a distance. No hay from an infested district should be allowed to be scattered in or near meadows in localities where the insect is not already present.

(9) Natural enemies consist so far as at present known of spiders, the predacious damsel bugs, *Reduviolus ferus*, a tachnid fly, *Phorantha occidentis*, and an undetermined species and a species of fungus, *Entomophthora* sp.

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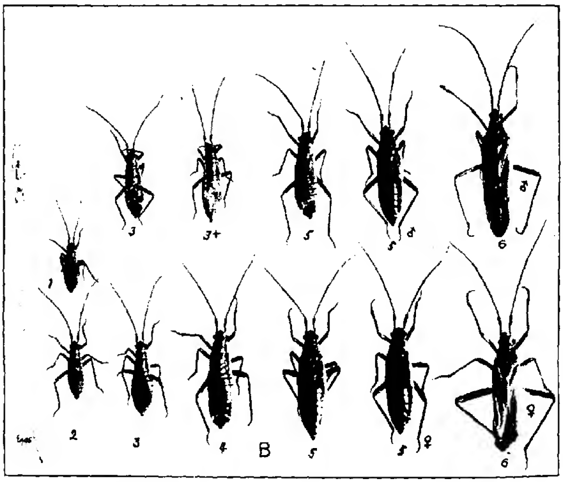
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PLATE 12

Miris dolabratus:

A.—*a*, Eggs in grass stem, all hanging in one direction; *b*, eggs in grass stem placed in opposite directions; *c*, same as upper part of *b*, more enlarged; *d* and *e*, eggs in clover stems. Photographed by Mr. Hammond.

B.—Eggs at left; *1*, first instar; *2*, second instar; *3*, third instar; *3*+, third instar more mature; *4*, fourth instar; *5*, fifth instar; *6*, adult male above, female below. Photographed by Mr. C. J. Drake.

ANGULAR-LEAFSPOT OF CUCUMBER: DISSEMINATION, OVERWINTERING, AND CONTROL

COOPERATIVE INVESTIGATIONS BETWEEN THE UNIVERSITY OF WISCONSIN AND THE BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE

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INTRODUCTION

The bacterial nature and the symptoms of the angular-leafspot of cucumber (*Cucumis sativus*) have been clearly described by Smith and Bryan (*15*).[2] Prior to this paper the disease had been reported in this country by Burger (*1-4*) and from Europe by Traverso (*16*) and Potebnia (*12*). The two latter writers accepted Burger's statement that the organism which caused the spots on the leaves was also responsible for a serious rotting of the fruit. The inoculation studies made by Smith and Bryan (*15*) showed that the bacterium which caused the leaf-spotting was unable to produce a soft-rotting of the fruit. Extensive tests by the writer have confirmed their finding in this regard.

The bacterial cause of the disease was determined independently by the writer in the summer of 1915, as set forth in a preliminary note (*5*). The morphological and physiological studies which were subsequently made of a strain of the causal organism isolated from a Wisconsin specimen gave results essentially in agreement with those reported by Smith and Bryan (*15*). The name given to the organism by these writers is "*Bacterium lachrymans*." According to Migula's system of classification it would be called "*Pseudomonas lachrymans*."

The damage caused by the angular-leafspot can not be accurately estimated. It varies greatly with differing weather conditions, but enough weather favorable for the disease prevails each year to make the injury of considerable importance (Pl. 13, A). The writer's ideas as to the destructiveness are based mainly on his field experience in Wisconsin and adjoining States during three summers, together with more limited observations in Virginia and southern California.

It is when the disease appears in a field early in the summer that the greatest damage results, as would naturally be expected. Young plants

[1] The writer wishes to express his appreciation to Dr. L. R. Jones, of the Wisconsin Experiment Station, for helpful interest and advice in the prosecution of the work and to thank Mr. W. W. Gilbert and Dr. M. W. Gardner, of the Bureau of Plant Industry, for helpful suggestions and cooperation.

[2] Reference is made by number (italic) to "Literature cited," p. 220.

are often so severely attacked that stunting results (Pl. 13, B). A few scattered plants in a field or nearly all may be so affected, depending chiefly on the meteorological conditions. Some observations on the extent of the injury by angular-leafspot in a representative locality may here be noted to give a more definite idea of the damage which it causes. At Ripon, Wis., in the summer of 1914, sixteen cucumber fields were under observation. The disease appeared in seven of these while the plants were in the seedling stage, and by the middle of the season, August 11, it had resulted in the severe spotting of approximately 25 per cent of the leaves. On August 11 the disease was also present in three of the nine other fields, but in these it had been introduced only a short time and had not yet become generally distributed. A survey of the same locality on August 15, 1915, indicates how widespread the disease may become, especially when the fields are close together. On that date 28 out of 35 fields visited were found to be infested. A later visit revealed even further distribution.

The losses in the regions where cucumbers are grown for pickling purposes result mainly from the decrease in yields due to the destruction of leaf surface, but it seems quite probable that in other sections, as has been pointed out by Burger (*1, 2*) for Florida, where cucumbers are grown for "slicing" purposes, and so must be shipped to distant markets or kept in storage for considerable lengths of time, an additional loss may come from the secondary soft-rotting of the fruit. Limited observations by the writer indicate that in California the soft-rotting of the fruit as an indirect result of the angular-leafspot may cause some loss. The bacterium causing the leafspot does not directly cause the fruitrot, but through the wounds which it makes on the fruit softrot organisms are frequently able to gain entrance.

The widespread distribution of angular-leafspot and its frequent occurrence give it a place among the major diseases of the cucumber. The aggregate loss which it entails probably exceeds that caused by some of the other diseases which are more destructive in limited areas. In America this disease has been reported from Florida by Burger (*1-4*), and from Connecticut, Indiana, Michigan, New York, and Wisconsin and the Canadian Provinces of Ontario and Quebec by Smith and Bryan (*15*). To this list of regions where it is known to occur may now be added California, Colorado, Illinois, Iowa, Minnesota, and Virginia. That it is probably widely distributed in Europe is indicated by the fact that Traverso (*16*) reported it from Italy, and Potebnia (*12*) recorded its occurrence in Russia. The wide distribution of the disease is a fact that should be expected in view of the evidence to be presented that the causal bacteria are seed-borne and in view of the general occurrence of the trouble in seed-growing localities.

PRELIMINARY CONSIDERATIONS

The chief purpose of this paper is to present evidence bearing on the phases of the problem which are of direct economic significance. Certain other parts of the work which has been done have yielded results worthy of record; and, since some of these results are pertinent to the questions of dissemination, overwintering, and control, they may appropriately be presented before passing to the consideration of the latter points.

DESICCATION

Many questions in regard to the dissemination and overwintering of the causal organism of angular-leafspot depend on its sensitiveness to desiccation. The organism has been shown by repeated tests to be relatively sensitive to drying on glass. With a 3-mm. platinum loop drops were transferred to carefully cleaned cover glasses from 36-hour cultures in beef bouillon and in cucumber-leaf decoction from the leaf exudate and from a suspension in distilled water of the organisms from freshly invaded tissue. None of these showed viable organisms after four days' drying at room temperature. Smith and Bryan (*15, p. 470*) found that the organisms from a young bouillon culture when dried on glass were viable after 21 days. The variance in these results may possibly be due to some slight difference in methods, which may have made a difference in the time for which the bacteria were exposed to complete desiccation.

Freshly invaded fruit and leaf tissue dried in diffuse light at room temperature showed viable organisms after 3 and 10 days, but none were alive after 32 days.

Short periods of drying, four to five days, resulted in the death of all organisms on seed which had been disinfected with mercuric chlorid, washed thoroughly, and then wet with a young bouillon culture of *Bacterium lachrymans*. The fact, however, that the bacteria do survive long periods of desiccation on or in the seed is shown by the evidence to be presented under the discussion of overwintering.

The results of one test on culture media are here pertinent because they show that there are conditions under which the organisms may withstand long periods of drying. On February 2, 1916, six tubes of potato-dextrose agar, in each of which had been suspended approximately 0.5 gm. of powdered calcium carbonate, were slanted and inoculated. The purpose was to see if the life of the cultures might be prolonged by neutralizing with the carbonate the acid resulting from the growth of the organisms. In a dextrose-containing medium the bacteria ordinarily make a rapid growth for a short time and then all die, so that the tubes become sterile, usually within 10 days. The tubes in this test were set away at room conditions and, because of the low relative humidity of the laboratory air, rapidly dried out. Before they dried com-

pletely, which required nearly two months, an abundant growth of the organisms had been made. On November 8, 1916, the dry remains of the agar, carbonate, etc., from three of the tubes were transferred to tubes of bouillon. Growth occurred in all three tubes thus inoculated, and in each case the identity of the organism was established by inoculating cucumber plants. Two of the remaining three tubes were similarly tested on February 9, 1917. Growth resulted in each case, and inoculations again proved that the clouding of the bouillon was due to the angular-leafspot organism. Since all other evidence is opposed to the possibility of the formation of spores, the writer is inclined to explain the survival of some of the bacteria in these tubes by assuming that they were protected from complete desiccation.

THERMAL RELATIONS

The thermal death point of the angular-leafspot bacterium is between 49° and 50° C. Tests were made in 10-cc. portions of beef bouillon in thin-walled test tubes at 46°, 48°, 49°, 50°, 52°, and 55°. Ten minutes' exposure at 46° must have killed a large proportion of the organisms, because growth in tubes so exposed was much slower in appearing than in the unheated controls. In each test some but not all of the tubes exposed at 49° C. showed no growth. In none of the tests did growth occur in tubes exposed at 50° or temperatures above that point.

An interesting contrast between the relation of temperature to angular-leafspot and its relation to the bacterial-wilt of cucumber was brought out at Madison, Wis., in 1916. The maximum temperature as recorded by the United States Weather Bureau there averaged 36.7° C. (98° F.) for the five days July 26 to 30, inclusive. The highest temperature at the Weather Bureau Observatory was 38.3° C. (101° F.), but in direct sunlight and near the ground undoubtedly the temperature was higher. This unusually hot weather did not appreciably check the development of angular-leafspot, which reached its maximum development within about 10 days thereafter, but it practically stamped out the bacterial-wilt. Smith (*14, p. 209*) accounts for the bacterial-wilt having been found only in cool climates on the basis of the low thermal death point, 43° C., of the causal organism.

The relation of temperature to growth in artificial media has been found to agree with the report of Smith and Bryan (*15, p. 470*), and so need not be given in detail.

The sensitiveness of the bacteria to freezing was tested by exposing them in different media in glass test tubes outside a north window during a period of low temperatures in the winter of 1916–17. Dilute suspensions of the bacteria in distilled water, freshly-inoculated tubes of beef bouillon, beef bouillon with approximately 2 per cent of sodium chlorid, and 24-hour agar slope cultures were exposed. During the first 9 days of the

exposure the highest temperature was 0° C. (32° F.), the lowest −25.5° C. (−14° F.), and the average daily mean was −15.5° C. (4.1° F.). One tube of each medium was taken in after 24 hours and longer periods and thawed slowly in cold water. In the salt bouillon all the bacteria were dead after 24 hours. In bouillon without salt all were dead after 60 hours. No test was made of the suspension in distilled water after the 60-hour interval, but no colonies developed in plates poured from one of the other tubes melted after 4 days. On the agar some of the organisms were alive after 6 days, but after 17 days all were dead. The sensitiveness to freezing was undoubtedly increased by the sodium chlorid in the bouillon.

Smith and Bryan (*15, p. 471*) reported freezing the organisms for 15 minutes in bouillon by means of salt and pounded ice. That exposure resulted in the death of nine-tenths of the bacteria. .

SENSITIVENESS TO GERMICIDES

Tests of the sensitiveness of the organisms to formaldehyde, copper sulphate, and mercuric chlorid were made. The dilutions of formaldehyde were made up by volume from the 40 per cent formaldehyde solution known commercially as formalin. The copper-sulphate and mercuric-chlorid solutions were made up 1 to 1,000 by weight and the desired dilutions made from these. Exposures were made in all cases by transferring a 3-mm. loop of a young bouillon culture to 10-cc. portions of the dilutions in vials floated on a water bath at 25° C. Tubes of melted agar were inoculated in duplicate or triplicate by a 3-mm. loop transfer from each vial after an exposure of 10 minutes.

The test with formaldehyde resulted in the death of all organisms exposed to a dilution of 1 to 10,000, of nearly all in the 1 to 100,000 dilution, and of apparently none in the 1 to 500,000 dilution. The tests with copper sulphate and mercuric chlorid were repeated twice. With the copper sulphate the results did not agree throughout, but in all cases all organisms were killed or prevented from developing by the 1 to 100,000 dilution. There were no colonies, or a strikingly smaller number than from the controls, in the plates poured from the 1 to 500,000 dilution. All organisms were killed by exposure to dilutions of mercuric chlorid of 1 to 1,000,000.

The sensitiveness of the organism to copper sulphate was tested by Smith and Bryan (*15, p. 474*). Their results show a slightly less marked sensitiveness to this chemical than was found in the tests made by the writer. The temperature at which their exposures were made was not stated.

PLANTS ATTACKED

Little attention had been previously given to the question of the host range of the disease or to the question of variations in susceptibility or resistance to the disease in the case of the different types of cucumbers.

Because of the bearing which these questions might have on distribution, overwintering, and control, 12 horticultural varieties of cucumbers and a large number of other cucurbits were tested as to susceptibility to angular-leafspot. The varieties of field cucumbers and the other species and varieties of cucurbits which are listed in the following table were grown in a cucumber field thoroughly infested with angular-leafspot, where they were under the most favorable conditions for infection. The varieties of forcing cucumbers were tested by inoculation in the greenhouse.

Plants exposed to angular-leafspot infection

PLANTS ATTACKED

1. Cucumber (*Cucumis sativus*), 12 horticultural varieties:
   - Davis Perfect.
   - Chicago Pickling.
   - Boston Forcing.
   - Early Russian.
   - Giant Pera.
   - Japanese Climbing.
   - Heinz Muscatine.
   - Lemon.
   - Thorburn's Everbearing.
   - Rollison's Telegraph.
   - Vaughan's Prolific Forcing.
   - White Spine Klondyke.
2. West Indian gherkin (*Cucumis anguria*).
3. Mandera gourd (*Cucumis acutangulis*).
4. Hedgehog gourd (*Cucumis dipsaceus*).
5. Calabash gourd (*Lagenaria vulgaris*).
6. *Bryanopsis laciniosa*.

PLANTS NOT ATTACKED

1. Balsam-apple (*Momordica balsamina*).
2. Balsam-pear (*Momordica charantia*).
3. Squirting cucumber (*Momordica elaterium*).
4. Pomegranate melon (*Cucumis melo* var. *dudaim*).
5. *Cucumis grossulariaeformis*.
6. Muskmelon (*Cucumis melo*), 11 varieties.[a]
7. Snake melon (*Cucumis melo* var. *flexuosus*).
8. Wild cucumber (*Echinocystis lobata*).[b]
9. Watermelon (*Citrullus vulgaris*), 2 varieties.[a]
10. Citron (*Citrullus vulgaris*).
11. Japanese crookneck squash (*Cucurbita moschata*).
12. Hubbard squash (*Cucurbita maxima*).[a]
13. Turban squash (*Cucurbita maxima*).
14. Summer squash (*Cucurbita pepo* var. *condensa*).
15. Pumpkin (*Cucurbita pepo*).[a]
16. Gourd (*Cucurbita pepo* var. *ovifera*).
17. *Trichosanthes colubrina*.

In the case of the plants other than cucumber attacked the causal organism was isolated from diseased spots and identified by inoculating cucumbers. Stained sections from paraffin-embedded material showed bacteria within the tissue of the leafspots in all cases except that of the West Indian gherkin, which was unquestionably subject to the disease.

STOMATAL MOVEMENT AND INFECTION

The fact that leaf infection took place through the stomata was reported by Smith and Bryan (*15, p. 469*), but they gave no discussion of the conditions necessary for infection. Practically all of the earlier inoculations made by the writer were performed in the evening, after dark,

[a] Greenhouse inoculations also gave negative results.
[b] The wild cucumber plants were not in the experimental plots, but grew near by and were artificially inoculated.

because the sensitiveness of the organism to sunlight was known and because moisture, such as dew on the leaves, was thought to be the most important factor in infection. A few infections were nearly always obtained in this way, but the number was consistently smaller than occurred on leaves naturally infected. A suggestion that the factors limiting the number of leaf infections were in some way involved with the time of inoculation was obtained when, from a series of inoculations made in the field at intervals of 2 and 4 hours during the day and night of a 24-hour period, more abundant infections resulted from the inoculations made during the day. Evidence that infection occurs more abundantly when inoculations are made during the day was confirmed by other tests.

The idea that during the process of photosynthesis enough oxygen was given off through the stomata to exert a chemotactic action on the causal bacteria was first conceived as a possible explanation of the different results from night and day inoculations. The hypothesis was abandoned after experimental tests. Plants which were kept in darkness for 24 hours before and after inoculation became infected to about the same extent as the controls.

The idea that stomatal movement might be a factor was next hit upon. Pool and McKay (*11*) found that there was a relation between stomatal movement in sugar-beet leaves and infection by *Cercospora beticola*. This fact suggested that in the case of the disease under consideration a similar relation might hold true. To study the behavior of the stomata the method described by Lloyd (*10*) of direct visual observation of the stomata *in situ* was utilized. It was found that the stomata on the lower surfaces of the leaves were generally open during the day and closed at night. The movement of the stomata on the upper surfaces was not always the same as those on the undersides, but this fact is of no special significance here. It was then found by repeated tests that inoculations on the under surfaces made in the morning, when the stomata were observed to be open, gave much more abundant infections than did similar inoculations made at night, when the stomata were seen to be closed (Pl. 14). The following table gives a comparison of the number of infections from night and day inoculations. The two plants used were of the same age, each having seven leaves at time of inoculation. Leaf 7 is the youngest leaf of each. They were treated similarly except for time of inoculation.

Time inoculated.	Leaf 3.	Leaf 4.	Leaf 5.	Leaf 6.	Leaf 7.
7.30 p. m.	0	0	5	48	45
10.00 a. m.	16	43	49	97	50

On the plant inoculated in the evening the youngest leaves, No. 6 and 7, showed many more infections than did the older leaves, and this has been repeatedly found in other inoculations. Why this difference in in-

fection of leaves of different ages occurs is a matter of conjecture, but it is thought to be associated with the fact that younger tissues are more susceptible. Probably the relatively small number of organisms which retain their motility are able, when the stomata open, to establish themselves in the younger leaves, but are not able to gain a foothold in the older tissues.

The closure of the stomata may mechanically exclude the bacteria or may interfere with stimuli which attract them into the interior of the leaves. No attempt has been made to determine this point, but the first theory seems to the writer the more plausible.

FRUIT INFECTION

Fruit infection occurs naturally without wounds. Stomatal infection (fig. 1) has been demonstrated in fruit artificially infected without wounding. Burger's (*4*) description of the effect on the fruit is accurate in part, but the softrot which he emphasizes results from organisms other than the species causing the small, circular, localized spots on the fruit, characteristic of angular-leafspot infections. The circular spots are at first water-soaked in appearance. Later their centers become whitened, owing to a cracking and drying out of the tissues (Pl. 16, B). In fruit, as well as leaf tissue, the bacteria have been seen only in the intercellular spaces.

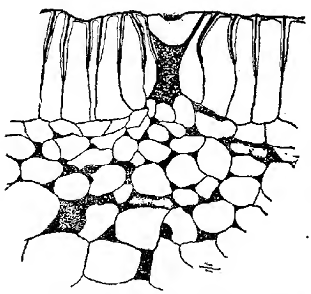

Fig. 1.—Cross section of epidermal portion of cucumber fruit fixed eight days after inoculation with *Bacterium lachrymans*, showing presence of bacteria in stoma and tissues below.

DISSEMINATION

The means by which the disease is spread have been given a good deal of attention because of the possible bearing which these might have on remedial measures. Some of the observations and experiments may throw light on other and similar bacterial diseases.

BY RAIN AND WIND

That the important relation of rainy weather to the progress of angular-leafspot, a factor previously observed, was principally in the dissemination of the causal organisms was made clear in the summer of 1916. Healthy potted plants which had been placed outside of the greenhouse and at a distance of 4 feet from infected plants became diseased after a

rainy period. Experimentally infection was secured by placing recently infected leaves on the ground beneath healthy plants on a day when there were frequent showers. In the fields at Madison newly infected spots appeared in abundance within five or six days after heavy rains, especially the rains of July 19 and August 3–5. Rain must fall at relatively frequent intervals to be effective in spreading the disease. Prolonged rainless periods check the development of the disease to a great degree, especially if accompanied by high temperatures (Pl. 15, C).

The importance of rain in the development of the disease was clearly shown at Ripon, Wis., in 1914. Owing to favorable rainy weather early in the season angular-leafspot spread throughout certain fields. Two of the infected fields at Ripon and one in a neighboring locality, which were visited on August 11 and 12, presented a striking appearance.

The vines were so grown together as to nearly cover the ground, but the centers of the rows were clearly marked by the old, angular-spotted leaves in contrast with the healthy green of the later growth which had developed after the last heavy rain.

Further evidence regarding the importance of rain in relation to the development of the disease was furnished by a comparison of conditions at Madison and Ripon, Wis., in 1916. The striking difference in the amount and distribution of rainfall for the two places during the month of July can be seen in Table I.

TABLE I.—*Dates and amounts of rainfall at Madison and Ripon, Wis., in July, 1916*

Day of month.	Precipitation (inches).	
	Madison.	Ripon.
1	0.00	0.13
12	.33	.11
16	.90	.00
19	1.21	.00
20	.19	.00
22	.03	.00
26	.00	.02
Total	2.66	.26

The time of planting and the earlier weather conditions were similar, and so it is highly probable that the disease appeared in both localities at about the same time, noted first at Madison on July 3. At the end of the month the disease was widespread and affecting leaves of all ages in the Madison fields, while at Ripon only the older leaves at the hill centers showed the angular spots.

The relation of wind to dissemination by rain spattering has not been studied experimentally, but the comparison of the way the disease spread in differently situated fields throws some light on the question.

One field, which we may call field A (fig. 2), was on the southeast slope of a hill and surrounded by trees so that it was well protected from wind, especially northwest wind. Field B was on the west slope of another hill and freely exposed to wind. The original centers of angular-leafspot in B were on the north side near the top. Thunder showers on July 12, 16, and 19 were accompanied by high winds from the north and west. On July 29 it was noted that in field A the infested areas were strikingly more delimited than those in field B. The difference could be explained only as a result of the difference in exposure to winds.

Another field was situated on a freely exposed west slope, and its rows ran with the hill, east to west. No notes on disease distribution there were taken until August 4. On that date there was a center of abundant

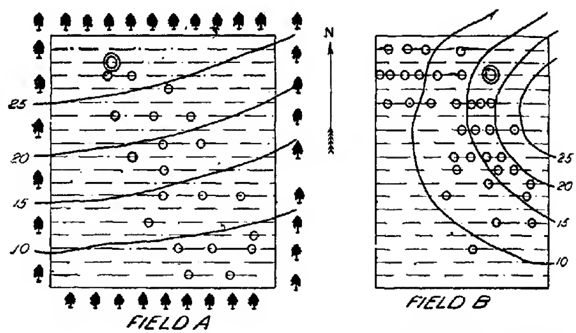

FIG. 2.—Diagrams of cucumber fields to show relation of wind and drainage water to angular-leafspot dissemination. Double circles indicate the location of original centers of infection prior to storms. Small circles represent secondary infections due to storms. In field A drainage water only was an important factor because of protection from wind while in the freely exposed field B wind also played a part. Contour lines indicate elevation above level of Lake Mendota, Madison, Wis. Broken lines show the direction of the rows. For full explanation see text.

infection in the seventh row from the north side and scattered infections in all of the 16 rows south of it. North of this badly infested area the adjacent row, the sixth, showed a very small number of infections, and the 5 others were entirely free from the disease. Obviously the northwest winds had played an important part here also in spreading the disease. Faulwetter (7) has shown that wind in connection with rain is an important factor in the spread of a similar bacterial disease, the angular-leafspot of cotton.

The fact that the thundershowers mentioned as resulting in a marked spread of angular-leafspot occurred during the daytime supports the inference which may be drawn from the facts regarding the relation of stomatal movement to infection, that rains which occur in the daytime are more effective in the spread of the disease than are those occurring at night.

BY DRAINAGE WATER

Evidence concerning the distribution of the causal organism by drainage water during rains was afforded by comparing developments in fields A and B which were mentioned in the preceding section. The rows in field A ran across the hillside, while in B they followed the direction of the slope (fig. 2). After the rains of July 12, 16, and 19 the disease appeared in field B throughout the length of the rows in which it had been noted earlier and in plots below them where disinfected seed had been planted. In field A, however, the spread of the disease was not mainly along the rows but rather crossed the rows, following the path of the drainage water. The supposition is that the organisms were carried by the drainage water and from it were spattered by the rain to the healthy plants. Dissemination by drainage water has been noted before with fungus diseases—for example, cabbage-yellows by Jones and Gilman (*9*)—but, so far as is known to the writer, no evidence has before been published in regard to its significance in the case of a bacterial disease.

Attempts to prove that drainage water carried the causal organisms were made on two occasions late in the summer. Samples of drainage water caught during rains at the lower edges of infested fields were taken to the greenhouse and sprayed on healthy plants. The negative results are not surprising in view of the fact that few new infections developed in the fields where the water was caught, and that negative results from attempts to isolate the bacterium from beetles from these same fields also indicated that a large proportion of the bacteria had been killed as a result of the long, preceding period of dry weather.

BY PICKERS

The spread of diseases due to fungi has been attributed to pickers—for example, bean anthracnose, by Whetzel (*17*)—but, so far as the writer is aware, no such fact has been demonstrated for a bacterial disease. Experiments in the case of the cucumber angular-leafspot have shown that the disease may be spread by pickers if picking is done when the exudate is present on the infected leaves. On August 8 and 9, 1916, the matter was tested as follows: At 5.30, 7.30, and 8 o'clock on the morning of the first day and at 8 a. m. on the second day two or three leaves in each case were inoculated by rubbing with the hands (as is done by pickers) after having first rubbed them through the exudate on diseased leaves. In all four cases inoculated leaves became infected (Pl. 15, B), while the uninoculated controls remained healthy.

Picking is, of course, frequently done early in the morning and on rainy days when the leaves are wet and the bacterial exudate is abundant. Numerous observations show that the spread of the disease in the way described in the preceding paragraph often results. The most obvious

of the cases that have come under the writer's notice seems worth mentioning in detail. At Princeton, Wis., a patch of cucumbers of seven rows was visited on August 12, 1916. In the middle of the third row, counting from the north side, there was a circular area of diseased leaves, badly shattered by the rain of August 10. West of this area of shattered leaves no new infections were evident. East of this area, however, there were numerous recent infections, and the number of these varied nearly inversely as the distance from the original center. The location of diseased leaves and the position of the spots on them corresponded to observations on dissemination by pickers made in other places. When passing the patch later in the day the owner was seen starting to pick on the west end of the first row, so that when he would come to pick the third row he would be working eastward (fig. 3). Evidently he must have followed the same course when the bacterial exudate was abundant enough to thoroughly contaminate his hands.

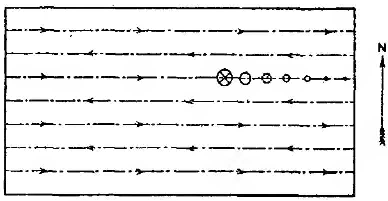

FIG. 3.—Diagram of cucumber field to illustrate picker dissemination of angular-leafspot. ⊗=original center, O=new infections, arrows indicate direction the picker worked.

BY INSECTS

Cucumber beetles (*Diabrotica vittata* Fab. and *D. duodecimpunctata* L.) have repeatedly been seen crawling over infected leaves and flying about the fields when the bacterial exudate was plentiful, as early as 5.30 a. m. Platings from these insects were made in only a few cases. That some of them would crawl through exudate and become contaminated seemed unquestionable. In one instance platings from a water blank, in which had been dropped three 12-spotted beetles, yielded the causal organism.[1] Bees have been observed visiting the plants as early as 7.30 a. m. and have been seen to occasionally brush against exudate-bearing leaves.

The carrying of the causal bacteria from one part of a field to another by insects is no doubt significant, but in view of the other ways in which the local spread is accomplished it is far less important than is the dissemination of the organism by the same agency from diseased to healthy

[1] The organism in this case was not tested as to pathogenesis, but was identified by colony characters on potato-dextrose agar. These typical colonies were more numerous than any other kind, and by transfers (unintentionally delayed until 10 days had passed) it was shown that the organisms were dead, as is true for the angular-leafspot organism with media containing dextrose.

fields. The evidence supporting the latter idea is observational. During 1916 six experimental fields were grown near Madison in the same vicinity with four privately owned commercial fields. The distances between the commercial and the experimental fields varied from about 30 rods to ½ mile. Angular-leafspot appeared in all six of the experimental plots early in July. It appeared in only three of the others and in these not until nearly the middle of August. Prior to this, in the forepart of the month, there was a period in which there was an abundance of bacterial exudate in the infested fields and when insects, especially the beetles, were very active. It is of interest here to note that in no case did the original center of infection in a private field develop at the edge, but rather in the interior of each of the three patches. It may be stated with confidence that during the time in question no one except Dr. M. W. Gardner and the writer visited both the experimental and commercial fields, and as these visits were made when the leaves were dry there seems little probability of the organisms having been transported by us. Comparable developments were observed at Ripon, Princeton, and Pittsville, Wis.

Closely correlated with picker dissemination and the probability of spread by insects is the relation of atmospheric humidity to the disease. Under conditions of high relative humidity, such as frequently prevail on summer nights, the invaded areas of the leaves take on clear-cut angular shapes and the bacterial exudate becomes abundant. (See Pl. 13, A). Such nights were those of August 8 and 9, 1916. The relative humidity, as recorded by a Friéz hygrograph, varied on those nights from 74 and 80 per cent, respectively, at 7 p. m. to 90 per cent, where it continued until 6 a. m. Observations in the early mornings showed abundant signs, as described, that conditions for the progress of the disease had been most favorable. In steam-heated greenhouses, with the relative humidity varying from 45 to 60 per cent, the disease develops poorly or not at all.

OVERWINTERING

The several ways in which the causal organisms of other bacterial diseases have been thought to pass the winter have been kept in mind in searching for evidence as to how the angular-leafspot bacterium overwinters.

SOIL

The sensitiveness of the organisms to freezing, as elsewhere recorded, renders doubtful the possibility of their living over the winter in the soil or in the débris of diseased vines in northern climates. A limited amount of work on this question indicates that the bacteria do not live for long periods in the soil. The question can not, however, be definitely settled until further study of it has been made.

INSECTS

The hypothesis that the bacteria may overwinter in or on the bodies of insects is here mentioned because it might seem plausible in view of the theory advanced with good evidence by Rand and Enlows (*13*) to account for the overwintering of the organism causing the wilt of cucurbits. No dependent relationship, such as has been found to exist between the wilt and cucumber beetles, has been observed in the case of angular-leafspot. Field observations in 1916 furnish some good negative evidence relative to the insect-overwintering theory. In one vicinity near Madison six cucumber fields on "new" land planted with seed from one source became diseased early, while four fields (planted with seed of a different source) on or very near land which had previously been planted with cucumbers did not develop the disease early. The early brood of beetles was fully as abundant on the four latter fields as on those six that became diseased early in the season.

SEED

The observations which formed the preliminary basis for the seed-overwintering theory have been printed before (*6*), but for the sake of bringing together all the pertinent evidence may be here repeated. In June, 1915, angular-leafspot was observed in abundance in a field south of Portsmouth, Va. The plants were developing their fifth and sixth leaves at the time. The field was on newly cleared land, surrounded by woods and at least 3 or 4 miles from the nearest cucumber patch. The evidence pointed strongly to the introduction of the organisms with the seed.

The developments in the fields near Madison in 1916 gave further evidence that the organisms are introduced with the seed. The six experimental fields previously mentioned were all on land which had not been planted to cucumbers for at least three years. Angular-leafspot appeared on seedlings in all six of these fields, and in three of them it was noted on the cotyledons. In the case of the four commercial fields near by which were planted with seed from another source the disease did not appear at all in one and not until late in the season in the other three. This evidence so strongly indicated that the bacteria live over winter on the seed that it seemed worth while to study the matter in the commercial seed fields. Accordingly the writer visited a large seed-producing center in Iowa and Dr. M. W. Gardner, because of his interest in the question in relation to cucumber anthracnose, visited a seed farm in Ohio. In one of the seed fields in Iowa the disease was widespread and, according to a hasty estimate, 25 per cent of the fruits were attacked. Dr. Gardner found spots on the fruits in the Ohio fields which he was reasonably sure were due to the angular-leafspot organism.

Since the fruit invasions are local and shallow, it is evident that the seed rarely, if ever, becomes attacked naturally. A study of the way the seed is thrashed, however, sheds further light on the way in which the seed may become contaminated.

The thrashing process practiced on the farms visited is probably in general use. It is begun by shoveling the whole fruits into a grinding machine which chops them up and allows the larger parts of the fruit pulp to be carried off on a rotating screen. The seed, the juice, and the smaller pieces of pulp fall through the screen and are drained into containers. This much of the process would doubtless afford ample opportunity for the organisms to reach the seed. The next step, however, probably increases the chances for the seed to become contaminated. The seed with the pulp and juice is left in the barrels with frequent stirring for a period of time varying usually from one to three days. The angular-leafspot organism doubtless multiplies rapidly in this well aerated mixture of juice and pulp unless conditions become unfavorable owing to the by-products of other organisms. After the material containing the seed has stood in the barrels for the time mentioned, it is poured into other containers and the seed separated out as well as possible by repeated washings with water. Then the seed is dried on shallow trays, at first in the sunlight and later indoors. The process of thrashing includes no step which would be likely to kill all the bacteria.

Seed for further study was sent to Madison from both the Iowa and Ohio farms. The details of some of the experiments performed with this seed and the results are here summarized.

Experiment of February 20, 1917.—Sixteen flats of sand were steamed at 7 pounds' pressure for one hour. Then each flat was planted with approximately 150 seeds from Iowa. Before touching the seed the hands were rinsed in 70 per cent alcohol as a precautionary measure. After the flats were planted they were wet down with water that had been boiled (cooled), and boiled water was used in all subsequent watering. None of the resulting seedlings were diseased.

Experiment of March 3, 1917.[1]—Fourteen flats of sand were steamed as before. The hands were disinfected with mercuric chlorid and alcohol. The trowel was treated with hot water. Twelve flats were planted with seed from the lot from Ohio, about 100 seeds to each flat. One flat was planted with seed from the 1915 supply which had been treated with 1 per cent formaldehyde for 20 minutes and another with seed from the same lot which had been treated with 1 to 1,000 mercuric chlorid for five minutes—these two for controls. The flats were covered with sterilized wire screen to protect them from mice and rats. The flats were watered with water (cooled) which had been boiled.

On March 19 the writer found four seedlings in one of the flats showing typical signs of angular-leafspot as they had been observed on seedlings artificially infected by planting inoculated seed and on naturally infected seedlings in the field (Pl. 15, A). The attacked seedlings were in two separated places—two affected seedlings next to each other in each case—and apparently one seedling had been infected from its neighbor in each instance. From a seedling from each of the two places the organism was isolated and used in pure culture inoculations to reproduce the disease. Stained sections

[1]Performed by Dr. M. W. Gardner in connection with his work on cucumber anthracnose.

of one of the spots on one of the cotyledons showed bacteria in the intercellular spaces. Two of the seedlings were preserved as herbarium specimens. On March 27 another infected seedling was noted in another flat. The organism was isolated and identified by inoculation as before.

Experiment of March 27, 1917.[1]—Fourteen flats of sand and four of heavily composted garden soil were steamed for one hour at 7 pounds' pressure. All but two were planted with the seed from Ohio. These two planted with seed treated in 1916 with mercuric chlorid and untreated seed from the 1916 supply, respectively. Precautionary measures taken as before. On April 4 a typically infected seedling was noted in one of the sand flats planted with the Ohio seed and on April 7 a well-advanced stage of the disease was discovered on a seedling in another sand flat of the Ohio seed. There was no doubt as to the cause of the lesions from the characteristic signs—viz, water-soaked tissue and white exudate residue. Platings from each of these seedlings gave an abundance of the typical colonies.

The results of these experiments and the fact that in Dr. Gardner's later tests of the Ohio seed in sterile damp chambers one seedling in each of two damp chambers developed the typical signs of the disease prove that the angular-leafspot organisms may live for at least seven months on the seed. There seems no reason to doubt but that they can survive for two months longer and infect the seedlings as field observations have indicated.

The use of seed as badly contaminated as the lot from Ohio was found to be, would have resulted in the early development of angular-leafspot in as large a proportion of the fields as occurred in 1916 in Wisconsin. From the Ohio lot approximately 3,500 seeds were planted with the precautions described. Seven, or a proportion of 1 to 500, of the resulting seedlings developed angular-leafspot. With this proportion or 0.2 per cent and the use of 2 pounds of seed per acre, as is usually practiced, there would be about 72 plants infected from seed-borne organisms to every acre of cucumbers.

As to how the organisms are protected on the seed so as to withstand the long period of desiccation there is no conclusive evidence. It seems most likely to the writer, however, that they get in at the micropylar end of the seed, and so are protected within the seed coat. The fact that the infections of the seedlings nearly always occur on the edge of the cotyledons near the point of attachment to the stem—the part of the cotyledons which is at the micropylar end—indicates that the bacteria are probably harbored beneath the seed coat (Pl. 16, A). It might be argued that, since on germination the attached ends of the cotyledons are the first to emerge, the portion which becomes infected is the first part which is exposed to organisms on the surface of the seed. This explanation, however, seems less probable to the writer than that the organisms are sheltered inside the micropyle. At any rate subsequent work by Gardner and Gilbert (*8*) has shown that the bacteria are so located that they can be killed by chemical treatment of the seed.

[1] Performed in cooperation with Dr. M. W. Gardner.

REMEDIAL MEASURES

The matter of finding some means of controlling angular-leafspot has been kept in mind in all the studies, especially in comparing cucumber varieties as to susceptibility, in observing the ways in which the disease is spread, in testing the sensitiveness of the organism to desiccation, to heat and chemical germicides, and in trying to determine how the bacteria are overwintered.

RESISTANT VARIETIES

Tests made in the field in 1915 and 1916 by growing the horticultural varieties (listed on page 206) where they were exposed to infection yielded no encouraging results. There was no marked difference in susceptibility between the varieties. No instance of individual resistance has been observed in all the fields which have been examined.

SANITATION

The evidence recorded under the section on dissemination by pickers justifies the recommendation that where feasible the picking of fields into which the disease has been introduced be done at times other than in early mornings or on rainy days when the bacterial exudate is abundant. In cases where it is necessary to pick over a partly diseased field under those unfavorable conditions it may be worth while to pick the healthy part of the field first.

The hope for the complete control of the insect pests, particularly the cucumber beetles, seems to be a thing for which there is little basis. The fact, however, as discussed under the consideration of dissemination, that there is good evidence that these insects are instrumental in spreading the disease from one field to another makes more urgent the need of finding better ways of holding them in check.

SPRAYING

Spraying experiments in which Bordeaux mixture (3-6-50) was the principal fungicide used, were under observation in Wisconsin during the summers of 1914, 1915, and 1916. Noticeable checking of the disease resulted each year. Yield results were in all cases so vitiated by factors other than the spraying, especially the mosaic disease, that comparisons of them were of little value. Furthermore, the disease did not develop in the most destructive way on the experimental fields. The data at hand therefore hardly justify a definite statement of the value of spraying for this disease, but, in the opinion of the writer, the practice would not in Wisconsin and neighboring States be generally profitable on a commercial scale. Several reasons have furnishd the basis for this conclusion. Because of the early appearance of the disease, spraying, to be most effective, would have to be started nearly as soon as the plants came up. Because of this need for beginning early and continuing the

spraying at frequent intervals throughout the season, the cost would probably be greater than could be compensated by the resulting increase in yield. Cucumber vines normally grow so rapidly that the intervals between spraying would have to be short in order that a considerable portion of the younger leaves would not be exposed to infection a good deal of the time. The fact, however, that the disease is mainly dependent on rain for dissemination and that long, rainless periods occur at irregular times would make it a hard matter to recommend a spraying schedule which would be economical.

Spraying where profitable because of other considerations has no doubt been of increased value because of the partial protection afforded from angular-leafspot damage.

Burger (*4*) reported beneficial results from spraying for this disease on the basis of a limited amount of spraying in one season. He found a decidedly smaller number of infected fruits in the sprayed than in the unsprayed plots, and reported that the leaves in the sprayed plots were healthier than those in the check rows. It is interesting to note, however, that his recorded yields show that in every case the total yield, including infected and healthy fruits, was greater from the check than from the sprayed plot. This fact may be correlated with the unsettled question of spray injury to cucumber.

The readiness with which angular-leafspot is spread by spattering of rain makes a spraying experiment, in which the check rows are parallel and adjacent to those sprayed, incomparable to the spraying of a whole field. This fact should be borne in mind when further spraying tests are made.

SEED TREATMENT

The evidence indicates strongly that the angular-leafspot organism overwinters principally on the seed. If this be true, the matter of controlling the disease is greatly simplified, especially from the standpoint of the industry of growing cucumbers for pickling. Some of the pickle companies grow their own seed, while others buy seed from seedsmen. All companies, so far as is known to the writer, furnish the seed to the growers with whom they contract to raise the cucumbers. There will be little difficulty, therefore, in getting the seed disinfected before it is distributed to the farmers, after a satisfactory method of treatment has been worked out.

Preliminary tests of treatments with hot water and with chemical disinfectants have been made. Seed has been treated as follows: Soaked in water at 50° and 52° C. for 10 minutes; in formalin (4 per cent) for 5 minutes and 2 minutes; in copper sulphate (1 per cent) 10 minutes and 5 minutes; and in mercuric chlorid (1 : 1,000) for 5 minutes and 2 minutes. These tests were run on such a small scale because of limited greenhouse space for testing germination that conclusions can not be drawn as to

the effectiveness of the treatments in killing the causal organism, but they do indicate that no important injury[1] to the seed from these treatments may be expected. Extensive field tests with treated seed and further field trials of disinfectants with special reference to injury to the seed are under way in Wisconsin, Michigan, and Indiana under the supervision of Mr. W. W. Gilbert and Dr. M. W. Gardner.

SUMMARY

Angular-leafspot of cucumber was first noted in Wisconsin in 1914 and its bacterial nature established in 1915. The disease is the same as that described by Smith and Bryan (*15*) and earlier reported by Burger (*2*), Traverso (*16*), and Potebnia (*12*).

The disease is probably world-wide in its distribution. Under favorable meteorological conditions it does a good deal of damage. Because of its widespread and frequent occurrence it should be ranked among the cucumber diseases of major economic importance.

Leaf infection is stomatal. Inoculations made at different hours showed that infection occurs chiefly during the day rather than the night. This is probably to be explained by the fact that the stomata are open during the day and closed at night.

Fruit infection is stomatal. The disease first appears there as small, localized, circular, water-soaked spots. The centers of the spots later become whitened, so that they are more readily noticed.

Rain is the most important means of dissemination, but pickers and probably insects play a part in this process.

The causal organism is sensitive to desiccation, is readily killed in artificial media by freezing, is killed in liquid media by an exposure for 10 minutes at 50° C., and is readily killed by dilute solutions of formaldehyde, copper sulphate, or mercuric chlorid. The sensitiveness of the organism to these chemicals is increasingly greater in the order mentioned.

There is substantial evidence that the causal bacteria overwinter with the seed.

No marked variation in resistance or susceptibility has been found among horticultural varieties of cucumbers. A few ornamental gourds are attacked by the disease. Attacks are limited to the cucurbits, and in that family no important crop plant other than the cucumber has been found affected.

Sanitary measures, such as precautions in picking and in control of insects, may be helpful. Spraying with Bordeaux mixture checks the disease, but is of doubtful value as a general commercial procedure in regions where spraying would not otherwise be practiced. Seed treatment offers the greatest hope of satisfactory control.

[1] In the subsequent field tests carried on by Gilbert and Gardner, the 4 per cent formalin treatment caused considerable injury to cucumber seedlings, resulting in marked rolling of cotyledons and retardation of growth. The mercuric chlorid treatment (1 : 1,000 for five minutes) has proved safe and effective (*8*).

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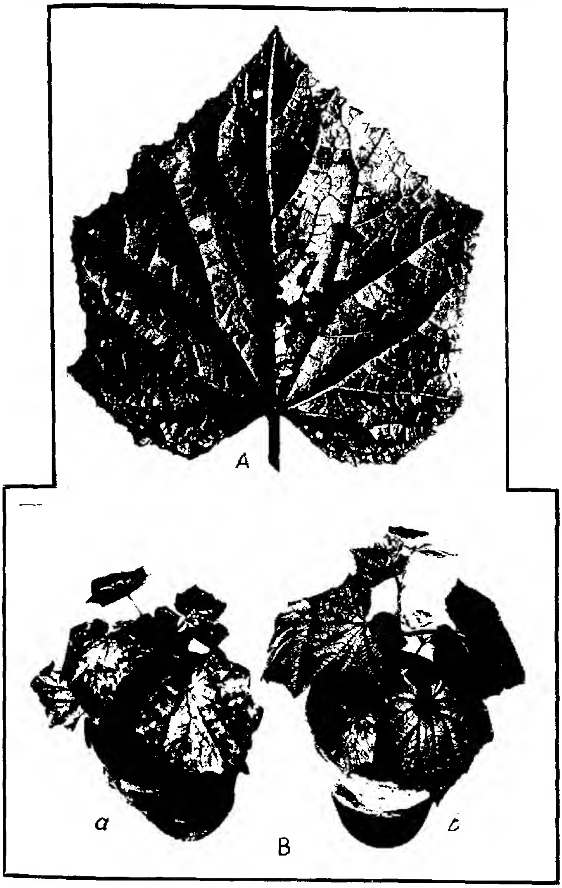
PLATE 13

A.—Cucumber leaf five days after inoculation with *Bacterium lachrymans*, showing severe infection. The dark, angular spots had a water-soaked appearance. Drops of bacterial exudate may be seen on some of the spots. Photographed by Mr. Fred R. Jones.

B.—Plant a, photographed seven days after inoculation with *Bact. lachrymans* shows considerable stunting as compared with the uninoculated control, plant b. Young plants as severely attacked have often been seen in the field.


A
a
B
b

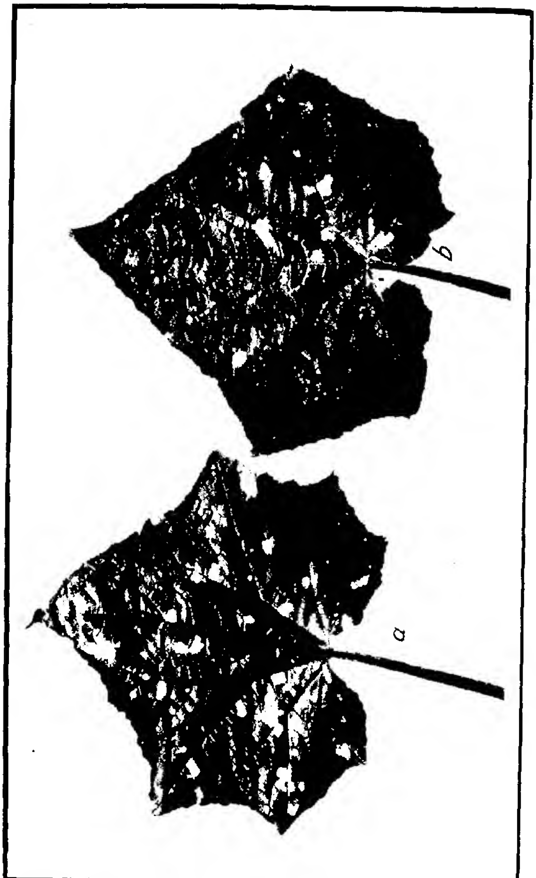

a
b

PLATE 14

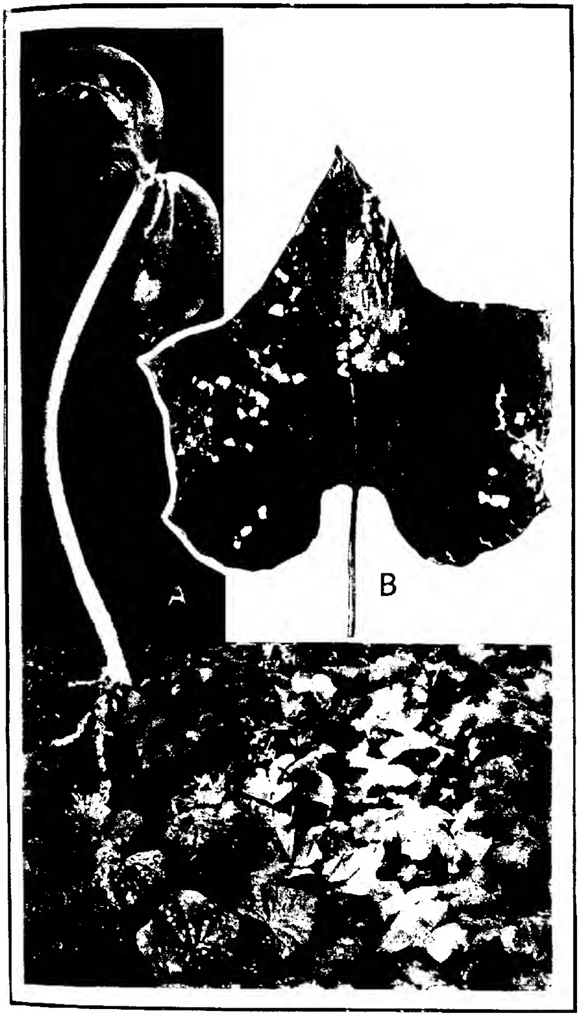
Stomatal movement in relation to infection. The cucumber leaves used in the experiment were of the same age and on similar plants. So far as possible, conditions of inoculation were similar except that leaf a was inoculated with *Bacterium lachrymans* at 9.15 a. m. and leaf b at 6 p. m.

PLATE 15

A.—Overwintering on seed: Natural infections on cotyledons of seedling grown in steamed sand from commercial seed which had been kept in storage for seven months after harvesting. Experiment of March 3, 1917. Enlarged about 1½ times.

B.—Picker dissemination. Infection resulting from inoculation of a cucumber leaf at 7.30 a. m. by rubbing with the hand immediately after touching diseased exudate-bearing leaves.

C.—Dissemination by rain. The older leaves in the center of the row were badly infected during a rainy period. Young leaves on the sides of the row which developed during a rainless period are comparatively free from the disease. Photographed by Mr. W. W. Gilbert.


A
B


a
b
A
B

PLATE 16

A.—Seedling infection resulting from seed inoculation with *Bacterium lachrymans*. Seeds were wet with a pure culture of the angular-leafspot organism and planted in sterilized soil. Note location of cotyledon infections. Photographed 14 days after planting.

B.—Cucumber fruit showing small, watersoaked, circular spots with white centers resulting from natural infections with angular-leafspot.